(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 733 709 A2

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
25.09.1996 Bulletin 1996/39

(51) Int. Cl.^{6}: C12N 15/54, C12N 9/10, C12N 15/70, C12N 1/21

(21) Application number: 95115423.6

(22) Date of filing: 29.09.1995

(84) Designated Contracting States:
BE CH DE FR GB IT LI SE

(30) Priority: 14.02.1995 JP 25253/95

(71) Applicant: TOYOTA JIDOSHA KABUSHIKI KAISHA
Aichi-ken (JP)

(72) Inventors:
- Ayumi, Koike,
c/o Toyota Jidosha K.K.
Toyota-shi, Aichi-ken (JP)
- Tokuzo, Nishino,
c/o Toyota Jidosha K.K.
Toyota-shi, Aichi-ken (JP)
- Shusei, Obata,
c/o Toyota Jidosha K.K.
Toyota-shi, Aichi-ken (JP)
- Shinichi, Ohnuma,
c/o Toyota Jidosha K.K.
Toyota-shi, Aichi-ken (JP)
- Takeshi, Nakazawa,
c/o Toyota Jidosha K.K.
Toyota-shi, Aichi-ken (JP)
- Kyozo, Ogura,
c/o Toyota Jidosha K.K.
Toyota-shi, Aichi-ken (JP)
- Tanetoshi, Koyama,
c/o Toyota Jidosha K.K.
Toyota-shi, Aichi-ken (JP)

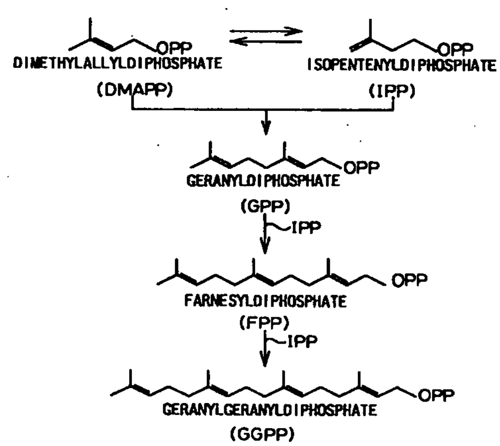
(74) Representative: Tiedtke, Harro, Dipl.-Ing.
Patentanwaltsbüro
Tiedtke-Bühling-Kinne & Partner
Bavariaring 4
80336 München (DE)

Remarks:
The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(54) Mutated farnesyldiphosphate synthase capable of synthesizing geranylgeranyldiphosphate and gene coding therefor

(57) A mutated farnesyldiphosphate synthase capable of synthesizing geranylgeranyldiphosphate and gene coding for said mutated enzyme, wherein the mutated enzyme is modified from a native farnesyldiphosphate synthase by mutation of a gene coding for a native farnesyldiphosphate synthase.

Fig.1



EP 0 733 709 A2

Description

BACKGROUND OF INVENTION

1. Field of Invention

The present invention relates to the mutated farnesyldiphosphate synthase capable of synthesizing geranylgeranyldiphosphate and a process for production thereof, as well as genes coding for said mutated enzymes and a process for isolation thereof.

2. Related Art

In nature there are various isoprenoid chain compounds comprising 5 carbon atom-basic structure, isoprene units, and these isoprenoid compounds play important roles for the life of various organisms. It is known that the chain-extension mechanism is catalyzed by a series of prenyltransferases which catalyze a series of catalytic reactions comprising sequential condensation of isopentenyldiphosphate (IPP) having 5 carbon atoms with its isomer dimethylallyldiphosphate (DMAPP). Among the isoprenoid compounds, farnesyldiphosphate (FPP) having 15 carbon atoms is positioned at a branching point in a biosynthesis pathway, from which various physiologically important start to geranylgeranyldiphosphate (GGPP) having 20 carbon atoms, to quinones, squalene, to steroids, farnesylated protein, dolichol etc.

Different prenyltransferases synthesize different isoprenoid compounds having different lengths. However, prenyltransferases have a common activity to condense an isoprenoid unit to extend the chain, and in fact, amino acids essential for the condensation are being clarified on the basis of homology of amino acid sequences of different prenyltransferases. However, the mechanism which determines the length of the isoprenoid compound have not yet clarified.

A biosynthesis pathway for geranyldiphosphate (GPP), farnesyldiphosphate (FPP) and geranylgeranyldiphosphate (GGPP) starting from an isoprenoid unit is shown in Fig. 1. In this biosynthesis pathway, the prenyltransferase which synthesizes farnesyldiphosphate is designated "farnesyldiphosphate synthase", and the prenyltransferase which synthesizes geranylgeranyldiphosphate is designated "geranylgeranyldiphosphate synthase".
Farnesyldiphosphate synthases are known in Bacillus thermophils (J. Biochem. 113, 355 - 363 (1993)), E. coli (J. Biochem. 108, 995 - 1000 (1990)), yeast (J.B.C. 265, 19176 - 19184 (1989)), rats (Mol. Cell. Biol. 7, 3138 - 3146 (1987)) and in humans (J.B.C. 265, 4607 - 4616 (1990)), and their amino acid sequences are also known.

On the other hand, geranylgeranyldiphosphate synthases are known in Rhodopseudomonas capusulata (J. Bacteriol. 154, 580 - 590 (1983)), Erwinia uredovora (J. Bacteriol. 172, 6704 - 6712 (1990)), Sulfolobus acidocaldarius (J.B.C. 269, 14792 - 14797 (1994)) etc.

However, it had not been known that an enzyme having geranylgeranyldiphosphate synthase activity can be obtained by mutation of farnesyldiphosphate synthase.

SUMMARY OF INVENTION

Accordingly, the present invention provides a novel geranylgeranyldiphosphate synthase obtainable by mutating a farnesyldiphosphate synthase and a process for production thereof, as well as gene system therefor and a process for isolation of the gene.

More specifically, the present invention provides a process for production of a gene coding for geranylgeranyldiphosphate synthase comprising the steps of:

(1) subjecting genes coding for a farnesyldiphosphate synthase to a mutagenesis;
(2) expressing the genes subjected to the mutagenesis, and
(3) selecting a gene which provides a geranylgeranyldiphosphate synthase.

The present invention further provides a gene coding for geranylgeranyldiphosphate synthase, an expression vector containing said gene, and a host transformed with said vector.

The present invention also provides a process for production of geranylgeranyldiphosphate synthase comprising expressing said gene, and geranylgeranyldiphosphate synthase obtainable by said process.

From another point of view, the present invention provides a geranylgeranyldiphosphate synthase having an amino acid sequence modified from an amino acid sequence of native farnesyldiphosphate synthase wherein the modification is deletion of one or more amino acids, addition of one or more amino acids, and/or replacement of one or more amino acids with other amino acids.

The present invention still further provides a gene coding for the above-mentioned geranylgeranyldiphosphate synthase, a vector, especially an expression vector comprising said gene, and a host transformed with said vector.

The present invention further provides a process for production of geranylgeranyldiphosphate synthase comprising the steps of cultivation said host, and purification the geranylgeranyldiphosphate synthase from the culture.

The present invention further provides a process for production of geranylgeranyldiphosphate or geranylgeranyol, comprising the steps of acting the present geranylgeranyldiphosphate synthase on isopentenyldiphosphate, dimethylallyldiphosphate, geranyldiphosphate or farnesyldiphosphate as a substrate.

BRIEF EXPLANATION OF DRAWINGS

Figure 1 represents a biosynthesis pathway for farnesyldiphosphate and geranylgeranyldiphosphate.

Fig. 2 shows the homology of amino acid sequences of farnesyldiphosphate synthase derived from different species. In this Figure, the sequences in the boxes A to E show regions having relatively high homology and which are expected to participate in enzyme activity.

Fig. 3 shows the homology of amino acid sequences of farnesyldiphosphate synthase derived from different species. In this Figure, the sequences in the boxes F and G show regions having relatively high homology and which are expected to participate in enzyme activity.

Fig. 4 shows a native amino acid sequence of farnesyldiphosphate synthase derived from Bacillus stearothermophilus (indicated as W.T), and the mutated points in amino acid sequences of the modified enzymes having geranylgeranyldiphosphate synthase activity (No. 1 to No. 4).

Fig. 5 schematically shows a process for construction of the present modified gene.

Fig. 6 is a profile of reversed phase TLC (developer: acetone/water = 9/1) showing products formed by acting the present enzyme on a substrate dimethylallyldiphosphate.

Fig. 7 is a profile of a reversed-phase TLC (developer: acetone/water = 9/1) showing products formed by acting the present enzyme on a substrate geranyldiphosphate.

Fig. 8 is a profile of a reversed phase TLC (developer: acetone/water = 9/1) showing products formed by acting the present enzyme on a substrate (all-E)-farnesyldiphosphate.

Fig. 9 is a profile of a reversed phase TLC (developer: acetone/water = 9/1) showing products formed by acting the present enzyme on a substrate (all-E)-farnesyldiphosphate.

DETAILED DESCRIPTION

Genes of the present invention can be obtained by subjecting a gene coding for a farnesyldiphosphate synthase to mutagenesis, expressing the genes subjected to the mutagenesis, and selecting a gene providing a protein having geranylgeranyldiphosphate synthase activity.

Genes coding for a farnesyldiphosphate synthase used in the present invention may be those of any origin. For example, farnesyldiphosphate synthases of E. coli, yeast, human, rat etc., as well as genes coding therefor are known, and amino acid sequences of these enzymes have high homology as shown in Fig. 2. Therefore, in addition to the gene derived from Bacillus stearothermophilus as described in detail, according to the present invention, any gene coding for an amino acid sequence having a high homology, for example, at least 20% homology with the amino acid sequence of farnesyldiphosphate synthase derived from Bacillus stearothermophilus can be used regardless of its origin. As such gene sources, for example, Bacillus stearothermophilus, E. coli, yeast, humans, rats etc. can be used.

The gene to be mutated is an RNA or DNA coding for a farnesyldiphosphate synthase and sensitive to treatment with a mutagen, and DNA is preferably used for to ease of handling, and especially a single-stranded DNA is preferred due to its high mutation ratio.

A single-stranded DNA can be easily prepared according to a conventional Procedure for preparing a single-stranded DNA, for example, by inserting a double-stranded DNA into a phage, introducing the phage into E. coli cells, culturing the E. coli cells and recovering the phage from the resulting lysate solution; or by introducing a desired double-stranded DNA into host cells, infecting the host cells with helper phage, culturing the host cells and recovering the phage from the resulting lysate solution.

Mutation of a gene can be carried out according to a conventional procedure for artificially mutating a gene. The mutation methods can be a physical method such as irradiation with X-rays, ultraviolet rays, etc., a chemical method such as treatment with a mutagen, a method of cis incorporation by DNA polymerase, a method using synthetic oligonucleotides etc. A chemical method is preferable for ease of operation and a high mutation ratio. As a mutagen, a nitrite, such as sodium nitrite, or the like can be used. To mutate a single-stranded DNA, a nitrite is preferable. Mutagenesis is preferably carried out at a nitrite concentration of 0.01 to 2M, for example, at about 0.1 to 1M, at a temperature of 20 to 30°C, for 10 to 120 minutes.

To select a gene coding for a protein having geranylgeranyldiphosphate synthase activity from the genes subjected to the mutagenesis, the gene subjected to the mutagenesis is inserted in an expression vector, the vector is introduced into host cells, the enzyme is expressed, and the expression product is tested for geranylgeranyldiphosphate synthase

activity. Geranylgeranyldiphosphate is converted to phytoene by a phytoene synthase, and the phytoene is converted to lycopene having red color by a phytoene desaturase.

Accordingly, for example, a gene coding for a phytoene synthase and a gene coding for phytoene desaturase are inserted into an expression vector, the vector is introduced into host cells such as E. coli cells, and further an expression plasmid comprising a DNA to be tested is introduced into said host cells, and the double transformed host cells are cultured. If the gene to be tested encodes a geranylgeranyldiphosphate synthase, and the geranylgeranyldiphosphate produced by the gene expression is converted to phytoene and further to lycopene, the cells are red-colored. Accordingly, a desired gene can be selected very easily and efficiently by selecting a red-colored colony.

The present invention provides a protein having geranylgeranyldiphosphate synthase activity, i.e., a geranylgeranyldiphosphate synthase, having an amino acid sequence modified from a native amino acid sequence of a farnesyldiphosphate synthase. Here, the modification of an amino acid sequence means replacement of one or a few amino acids with other amino acids, deletion of one or a few amino acids or addition of one or a few amino acids, or a combination of these modifications. The amino acid replacement is especially preferable. Regarding the number of amino acids to be modified, "a few amino acids" means usually about 15 amino acids, preferably about 10 amino acids, and more preferably about 5 amino acids. Namely, according to the present invention, the number of mutated amino acids is about 1 to 15, preferably about 1 to 10, and more preferably 1 to 5.

To determine the positions of modified amino acids, after the mutagenesis and the selection of a gene coding for a geranylgeranyldiphosphate synthase, a nucleotide sequence of the selected gene is determined, and an amino acid sequence is predicted from the determined nucleotide sequence, the predicted amino acid sequence of the modified enzyme is composed with the corresponding native amino acid sequence. Amino acid sequences thus determined of the modified enzymes are shown in Fig. 4.

In Fig. 4, the row indicated by the symbol W.T shows, by the one-letter expression, a native amino acid sequence of farnesyldiphosphate synthase of Bacillus stearothermophilus origin, and the rows Nos. 1 to 4 show representative amino acid sequences which acquired geranylgeranyldiphosphate synthase activity by amino acid replacement in the amino acid sequence of the farnesyldiphosphate synthase, wherein only the amino acids different from the corresponding amino acids in the native amino acid sequence of the farnesyldiphosphate synthase shown in the line T.W are indicated by the one-letter expression of amino acid.

The modified enzyme No. 1 has two mutations, i.e., the 81st position (Tyr→His) and 275th position (Leu→Ser); the modified enzyme No. 2 has two mutations, i.e., 34th position (Leu-Val) and 59th position (Arg→Gln); the modified enzyme No. 3 has two mutations, i.e., 157th position (Val→Ala) and 182nd position (His→Tyr); and the modified enzyme No. 4 has three mutations, i.e., 81st position (Tyr→His), 238th position (Pro→Arg) and 265th position (Ala→Thr). The amino acid sequences No. 1 to 4 of the above-mentioned modified enzymes and nucleotide sequences coding therefor are shown in SEQ ID NO: 1 to 4, and the native amino acid sequence and a nucleotide sequence coding therefor is shown in SEQ ID NO: 5.

In the present invention, the amino acid sequence farnesyldiphosphate synthase of Bacillus stearothermophilus origin was used as a specific example. However, as shown in Figs. 2 and 3, farnesyldiphosphate synthases have high homology among a wide spectrum of species covering those derived from the eukaryotes including humans and those derived from prokaryotes including bacteria. Therefore, the present invention can be applied to enzymes derived from various species to obtain novel geranylgeranyldiphosphate synthase.

As shown in Fig. 4, amino acid modification such as replacement occurs on the 34th, 59th, 81st, 157th, 182nd, 239th, 265th, and/or 275th positions of farnesyldiphosphate of Bacillus stearothermophilus. For enzymes from other species, it is expected that replacement at positions corresponding to the above-mentioned positions of the farnesyldiphosphate synthase of Bacillus stearothermophilus origin provides similar effects as that for the modified enzyme derived from Bacillus stearothermophilus. Therefore, the present invention can be applied to any farnesyldiphosphate synthases.

The present invention also relates to genes coding for the various geranylgeranyldiphosphate synthases derived from a farnesyldiphosphate synthase. These genes can be obtained by mutation of a gene coding for a corresponding native amino acid sequence. In addition, once the position of mutated amino acid is determined, a gene coding for the modified enzyme can be obtained by site-specific mutagenesis using a mutagenic primer. In addition, once an entire amino acid sequence is determined, a DNA coding for the amino acid sequence can be chemically synthesized according to a conventional procedure.

Genes coding for farnesyldiphosphate synthases used as starting materials to obtain the present genes have been cloned from various organisms, and therefore they can be used. For example, a gene of Bacillus stearothermophilus origin is described in J. Biochem. 113, 355 - 363 (1993), a gene of E. coli origin is described in J. Biochem. 108, 995 - 1000 (1990), a gene of yeast origin is described in J.B.C. 264, 19176 - 19184 (1989), a gene of rat origin is described in Mol. Cell. Biol. 7, 3138 - 3146 (1987), and a gene of human origin is described in J.B.C. 265, 4607 - 4614 (1990).

The present invention further provides recombinant vectors, especially expression vectors, comprising the abovementioned gene (DNA), recombinant host transformed with said vector, and a process for production of said enzyme using said recombinant host.

As an example, where E. coli is used as a host, it is known that there are gene expression control mechanisms which regulate transcription of DNA to mRNA, translation of mRNA to protein etc.

As promoter sequences which control the synthesis of mRNA, naturally occurring sequences such as lac, trp, bla, lpp, PL, PR, tet, T3, T7 et al., as well as mutants thereof, such as lacUV5, sequences prepared by fusing naturally occurring promoter sequences, such as tac, tra, etc. are known, and they can be used in the present invention.

As sequences which control the ability to synthesize a protein from mRNA, it is known that a ribosome-binding site (GAGG and similar sequence) and the distance between the ribosome-binding site and the start codon ATG are important. In addition, it is known that a terminator which directs the termination of transcription at the 3'-end (for example, a vector comprising rrnBT1T2 is commercially available from Pharmacia) influences the efficiency of protein synthesis in a recombinant host.

As starting vectors to prepare recombinant vectors of the present invention, those commercially available can be used. Alternatively, various vectors derivatized according to a particular purpose can be used. For example, pBR322, pBR327, pKK223-2, pKK233-2, pTrc99A etc. containing a replicon derived from pMB1; pUC18, pUC19, pUC118, pUC119, pTV118N, pTV119N, pHSG298, pHSG396 etc., which have been modified to increase copy number; pACYC177, pACYC184 etc. containing a replicon derived from p15A; as well as plasmids derived from pSC101, C01E1, R1 or F-factor, may be mentioned.

Further, in addition to plasmids, viral vectors such as λ phage, M13 phage etc., and transposones can be used for introduction of a gene. These vectors are described in Molecular cloning (J. Sambrook, E.F. Fritsch, J. Maniatis, Cold Spring Harbor Laboratory Press); Cloning vector (P.H. Pouwels, B.E. Enger-Valk, W.J. Brammer, Elsevier); and catalogs of manufacturers of vectors.

Especially preferable is pTrc99 (commercially available for Pharmacia) which has an ampicillin resistance gene as a selective maker, Ptrc and $lacI^q$ as a promoter and control gene, an AGGA sequence as a ribosome-binding site and rrnBT1T2 as a terminator, and therefore has a function to control an expression of a geranylgeranyldiphosphate synthase.

Introduction of a DNA coding for geranylgeranyldiphosphate synthase and if necessary DNA fragments having a function to control the expression of said gene into the above-mentioned vectors can be carried out using appropriate restriction enzymes and ligases according to a conventional procedure.

Such a recombinant vector can be used to transform a microorganism such as Escherichia coli, Bacillus etc. Transformation can be carried out according to a conventional procedure, for example by the $CaCl_2$ method, protoplast method etc. described, for example, in Molecular cloning (J. Sambrook, E.F. Fritsch, T. Maniatis, Cold Spring Harbor Laboratory Press), DNA cloning Vol. I to III (D.M. Glover, IRLPRESS).

Although methods for expression of the present gene in E. coli was described in detail, according to the present invention, a DNA coding for a geranylgeranyldiphosphate synthase is inserted into a conventional expression vector according to a conventional procedure, and the vector is used to transform a host, for example, prokaryotic cells such as various bacterial cells, lower eukaryotic cells for example single cell hosts, for example, yeast cells, or higher eukaryotic cells such as silk-worm. After transformation, the transformant is cultured to produce a geranylgeranyldiphosphate synthase, according to a conventional process.

When a transformant host such as E. coli is cultured, geranylgeranyldiphosphate synthase is intracellularly accumulated. To recover the geranylgeranyldiphosphate from the cultured host cells, the cells are treated physiologically or chemically, for example, with a cell lysating agent to lyze the cells. The cell debris is removed, and the supernatant is subjected to an isolation process conventional for purification of enzymes. The above-mentioned cell-lysing enzyme is preferably lysozyme, and the physical treatment is preferably treatment with ultrasonic radiation. When the supernatant is heated to a temperature of about 55°C, proteins intrinsic to E. coli are insolubilized and removed as an insoluble precipitate. To purify the enzyme, gel-filtration chromatography, ion exchange chromatography, hydrophobic chromatography, reversed chromatography, and affinity chromatography can be used alone or in combination. During the purification and isolation steps, the desired enzyme can be stabilized by addition of a reducing agent such as dithiothreitol, protecting agent against proteases such as PMSF, BSA etc., metal ions such as magnesium, alone or in combination.

The present invention further provides a process for production of geranylgeranyldiphosphate or geranylgeranyol. In this process, isopentenyldiphosphate, dimethylallyldiphosphate, geranyldiphosphate, farnesyldiphosphate may be used as substrates.

EXAMPLES

Next, the present invention is explained in more detail by means of examples, though the present invention is not limited thereto.

Example 1. Construction of mutated genes (Fig. 5)

The translation start codon in plasmid pFE15 (Japanese Unexamined Patent Publication (Kokai) No. 5-219761) containing a gene coding for farnesyldiphosphate synthase of Bacillus stearothermophilus origin was changed to ATG to obtain plasmid pEX11 (J. Biochem. 113, 355 - 363 (1993)) for overexpression of farnesyldiphosphate synthase, and the plasmid pEX11 was used in the following Examples. The mutation was carried out according to M. Myers et al. (Science, 229, 242 - 247 (1985)).

First, a farnesyldiphosphate synthase gene present in NcoI-HindIII fragment in pEX11 was removed, and inserted it into plasmid pTV118N (available from Takara Shuzo, Japan) to construct a plasmid, which was then introduced into E. coli cells. The transformed E. coli cells were cultured. With infection of a helper phage M13K07 (available from Takara Shuzo), pTV118N is converted to a single-stranded DNA and preferentially incorporated in phage particles and liberated out of cells. The culture was centrifuged to obtain a supernatant, from which the single-stranded DNA was recovered.

The single-stranded DNA thus recovered was subjected to mutation with sodium nitrite (concentration 1M or 0.2M) to introduce random mutation into the single-stranded DNA, which was then restored to a double-stranded DNA using AMV reverse-transcriptase XL (E.C.2.7.7.7). This farnesyldiphosphate synthase gene fragment was introduced into pTrc99A (available for Pharmacia) and pTV118N, and resulting recombinant plasmids were used to transform E. coli into which a phytoene synthase gene and phytoene desaturase gene had been previously introduced, and red colonies were selected. The principle of the selection is as follows.

The following screening method follows Ohnuma et al. (J. Biol. Chem., 269, 14792 - 14797 (1994)). E. coli harboring a plasmid pACYC-IB, into which crtB (phytoene synthase gene) and crtI (phytoene desaturase gene) of a phytopathogen Erwinia uredovora origin had been introduced, was transformed with the mutant plasmid. Note that at present it is believed that E. coli does not have a geranylgeranyldiphosphate synthase. If the mutant plasmid encodes geranylgeranyldiphosphate synthase activity, lycopene having red color is produced in E. coli cells by pACYC-IB resulting in formation of red-colored colonies. However, if the mutant plasmid does not encode geranylgeranyldiphosphate synthase activity, colonies are color-less. In this way, geranylgeranyldiphosphate synthase activity was easily detected by visual observation.

As a result of transformation of the E. coli cells with the mutant plasmid, red colonies were detected. The ratio of positive clones was 1.32×10^{-3} (10 colonies per 7,600 colonies) when the mutation was carried out using 1M $NaNO_2$, while the ratio of positive clones was 5.98×10^{-5} (one colony per 16,720 colonies) when the mutation was carried out using 0.2M $NaNO_2$, revealing that the higher the concentration of $NaNO_2$, the higher the positive ratio.

Among the positive colonies, four colonies were selected, and a nucleotide sequence of an enzyme-coding region in the plasmid was determined, and an amino acid sequence encoded by the nucleotide sequence was determined, for each positive clone. The result is shown in SEQ ID NOs: 1 to 4. In addition, these amino acid sequences were compared with the native amino acid sequence, and positions of the mutation are shown in Fig. 4.

Four mutated enzymes encoded by four mutant genes were further characterized.

Example 2. Production of mutated enzymes

E. coli transformed with the mutant plasmid was cultured in LB medium at 37°C overnight. The culture was centrifuged at 3,000 × G, at 4°C for 5 minutes to collect cells, which were then suspended in a buffer for sonication (50 mM Tris-HCl (pH 7.0), 10 mM 2-mercaptoethanol, 1 mM EDTA). The suspension was subjected to ultrasonic waves to disrupt the cells. The sonicate was centrifuged at 5,000 × g, at a temperature of 4°C for 20 minutes, to obtain a supernatant, which was then heated at 55°C for one hour to inactivate enzymes intrinsic to E. coli to obtain a crude enzyme extract.

To test the enzymatic activity of each mutant enzyme, reactions were carried out in the following reaction mixture.

Table 1

[1-14C]IPP (1 Ci/mol))	25 nmol
Allyl substrate (DMAPP, GPP, FPP)	25 nmol
$MgCl_2$	5 μmol
NH_4Cl	50 μmol
2-Mercaptoethanol	50 μmol
Tris-HCl buffer (pH 8.5)	50 μmol
Sample to be tested	proper quantity
Total	1 ml
Note: DMAPP: Dimethylallyldiphosphate GPP: Geranyldiphosphate FPP: Farnesyldiphosphate	

The reaction mixture was incubated at 55°C for 30 minutes, and the product was extracted with water-saturated 1-butanol, and radioactivity of the extract was counted by a liquid scintillation counter. In addition, the extract (butanol layer) was treated with an acid phosphatase and extracted with pentane. The extract was analyzed by TLC. The TLC analysis showed that the use of dimethylallyldiphosphate and geranyldiphosphate as an allyl substrate provides similar TLC patterns. Note that since the amount of each sample was adjusted so that the radioactivity is approximately same between the samples, the density of the band does not indicate specific activity.

The modified enzymes Nos. 1 and 4 produced an amount of geranylgeranyldiphosphate more than that of farnesyldiphosphate, and therefore it is considered that the modified enzymes Nos. 1 and 4 are suitable for the production of geranylgeranyldiphosphate. On the other hand, the modified enzymes No. 2 and No. 3 provided a small amount of geranylgeranyldiphosphate.

Where (all-E)-farnesyldiphosphate was used as a substrate (primer), (all-E)-geranylgeranyldiphosphate was formed. The results are shown in Figs. 6 to 9.

Specific activity and ratio of product (GGOH/FOH) are shown in Table 2.

Table 2

		Specific activity* (nmol/min/mg protein)	Ratio of product (GGPP/FPP)
Wild type		286	0
No. 1	pTV118N	0.293	18.4
	pTrc99A	0.253	6.28
No. 2	pTV118N	110	2.95×10^{-2}
	pTrc99A	83	2.54×10^{-2}
No. 3	pTV118N	143	1.65×10^{-1}
	pTrc99A	19.7	1.73×10^{-1}
No. 4	pTV118N	0.262	15.5
	pTrc99A	0.271	8.28

*DMAPP was used as substrate.

SEQUENCE LISTING

SEQ ID NO: 1

SEQUENCE LENGTH: 894

SEQUENCE TYPE: Nucleic acid

STRANDNESS: Double

TOPOLOGY: Linear

MOLECULAR TYPE:

SOURCE: Bacillus stearothermophilus

CHARACTERISTIC: Mutant (1) of DNA coding for farnesyldiphosphate synthase

SEQUENCE

```
ATG GCG CAG CTT TCA GTT GAA CAG TTT CTC AAC GAG CAA AAA CAG GCG        48
Met Ala Gln Leu Ser Val Glu Gln Phe Leu Asn Glu Gln Lys Gln Ala
                5                   10                  15
GTG GAA ACA GCG CTC TCC CGT TAT ATA GAG CGC TTA GAA GGG CCG GCG        96
Val Glu Thr Ala Leu Ser Arg Tyr Ile Glu Arg Leu Glu Gly Pro Ala
            20                  25                  30
AAG CTG AAA AAG GCG ATG GCG TAC TCA TTG GAG GCC GGC GGC AAA CGA       144
Lys Leu Lys Lys Ala Met Ala Tyr Ser Leu Glu Ala Gly Gly Lys Arg
        35                  40                  45
ATC CGT CCG TTG CTG CTT CTG TCC ACC GTT CGG GCG CTC GGA AAA GAC       192
Ile Arg Pro Leu Leu Leu Leu Ser Thr Val Arg Ala Leu Gly Lys Asp
    50                  55                  60
CCG GCG GTC GGA TTG CCC GTC GCC TGC GCG ATT GAA ATG ATC CAT ACG       240
Pro Ala Val Gly Leu Pro Val Ala Cys Ala Ile Glu Met Ile His Thr
65                  70                  75                  80
CAC TCT TTG ATC CAT GAT GAT TTG CCG AGC ATG GAC AAC GAT GAT TTG       288
His Ser Leu Ile His Asp Asp Leu Pro Ser Met Asp Asn Asp Asp Leu
                85                  90                  95
```

CGG CGC GGC AAG CCG ACG AAC CAT AAA GTG TTC GGC GAG GCG ATG GCC 336
Arg Arg Gly Lys Pro Thr Asn His Lys Val Phe Gly Glu Ala Met Ala
100 105 110

ATC TTG GCG GGG GAC GGG TTG TTG ACG TAC GCG TTT CAA TTG ATC ACC 384
Ile Leu Ala Gly Asp Gly Leu Leu Thr Tyr Ala Phe Gln Leu Ile Thr
115 120 125

GAA ATC GAC GAT GAG CGC ATC CCT CCT TCC GTC CGG CTT CGG CTC ATC 432
Glu Ile Asp Asp Glu Arg Ile Pro Pro Ser Val Arg Leu Arg Leu Ile
130 135 140

GAA CGG CTG GCG AAA GCG GCC GGT CCG GAA GGG ATG GTC GCC GGT CAG 480
Glu Arg Leu Ala Lys Ala Ala Gly Pro Glu Gly Met Val Ala Gly Gln
145 150 155 160

GCA GCC GAT ATG GAA GGA GAG GGG AAA ACG CTG ACG CTT TCG GAG CTC 528
Ala Ala Asp Met Glu Gly Glu Gly Lys Thr Leu Thr Leu Ser Glu Leu
165 170 175

GAA TAC ATT CAT CGG CAT AAA ACC GGG AAA ATG CTG CAA TAC AGC GTG 576
Glu Tyr Ile His Arg His Lys Thr Gly Lys Met Leu Gln Tyr Ser Val
180 185 190

CAC GCC GGC GCC TTG ATC GGC GGC GCT GAT GCC CGG CAA ACG CGG GAG 624
His Ala Gly Ala Leu Ile Gly Gly Ala Asp Ala Arg Gln Thr Arg Glu
195 200 205

CTT GAC GAA TTC GCC GCC CAT CTA GGC CTT GCC TTT CAA ATT CGC GAT 672
Leu Asp Glu Phe Ala Ala His Leu Gly Leu Ala Phe Gln Ile Arg Asp
210 215 220

GAT ATT CTC GAT ATT GAA GGG GCA GAA GAA AAA ATC GGC AAG CCG GTC 720
Asp Ile Leu Asp Ile Glu Gly Ala Glu Glu Lys Ile Gly Lys Pro Val
225 230 235 240

GGC AGC GAC CAA AGC AAC AAC AAA GCG ACG TAT CCA GCG TTG CTG TCG 768
Gly Ser Asp Gln Ser Asn Asn Lys Ala Thr Tyr Pro Ala Leu Leu Ser
245 250 255

CTT GCC GGC GCG AAG GAA AAG TTG GCG TTC CAT ATC GAG GCG GCG CAG 816
Leu Ala Gly Ala Lys Glu Lys Leu Ala Phe His Ile Glu Ala Ala Gln
260 265 270

CGC CAT TCA CGG AAC GCC GAC GTT GAC GGC GCC GCG CTC GCC TAT ATT 864
Arg His Ser Arg Asn Ala Asp Val Asp Gly Ala Ala Leu Ala Tyr Ile
275 280 285

TGC GAA CTG GTC GCC GCC CGC GAC CAT TAA 894
Cys Glu Leu Val Ala Ala Arg Asp His ***
290 295

SEQ ID NO: 2

SEQUENCE LENGTH: 894

SEQUENCE TYPE: Nucleic acid

STRANDNESS: Double

TOPOLOY: Linear

MOLECULAR TYPE:

SOURCE: Bacillus stearothermophilus

CHARACTERISTIC: Mutant (2) of DNA coding for farnesyldiphosphate systhase

SEQUENCE

ATG GCG CAG CTT TCA GTT GAA CAG TTT CTC AAC GAG CAA AAA CAG GCG 48
Met Ala Gln Leu Ser Val Glu Gln Phe Leu Asn Glu Gln Lys Gln Ala
5 10 15

GTG GAA ACA GCG CTC TCC CGT TAT ATA GAG CGC TTA GAA GGG CCG GCG 96
Val Glu Thr Ala Leu Ser Arg Tyr Ile Glu Arg Leu Glu Gly Pro Ala
20 25 30

AAG GTG AAA AAG GCG ATG GCG TAC TCA TTG GAG GCC GGC GGC AAA CGA 144
Lys Val Lys Lys Ala Met Ala Tyr Ser Leu Glu Ala Gly Gly Lys Arg
35 40 45

```
ATC CGT CCG TTG CTG CTT CTG TCC ACC GTT CAG GCG CTC GGC AAA GAC      192
Ile Arg Pro Leu Leu Leu Leu Ser Thr Val Gln Ala Leu Gly Lys Asp
             50                  55                  60
CCG GCG GTC GGA TTG CCC GTC GCC TGC GCG ATT GAA ATG ATC CAT ACG      240
Pro Ala Val Gly Leu Pro Val Ala Cys Ala Ile Glu Met Ile His Thr
 65                  70                  75                  80
TAC TCT TTG ATC CAT GAT GAT TTG CCG AGC ATG GAC AAC GAT GAT TTG      288
Tyr Ser Leu Ile His Asp Asp Leu Pro Ser Met Asp Asn Asp Asp Leu
                 85                  90                  95
CGG CGC GGC AAG CCG ACG AAC CAT AAA GTG TTC GGC GAG GCG ATG GCC      336
Arg Arg Gly Lys Pro Thr Asn His Lys Val Phe Gly Glu Ala Met Ala
            100                 105                 110
ATC TTG GCG GGG GAC GGG TTG TTG ACG TAC GCG TTT CAA TTG ATC ACC      384
Ile Leu Ala Gly Asp Gly Leu Leu Thr Tyr Ala Phe Gln Leu Ile Thr
        115                 120                 125
GAA ATC GAC GAT GAG CGC ATC CCT CCT TCC GTC CGG CTT CGG CTC ATC      432
Glu Ile Asp Asp Glu Arg Ile Pro Pro Ser Val Arg Leu Arg Leu Ile
    130                 135                 140
GAA CGG CTG GCG AAA GCG GCC GGT CCG GAA GGG ATG GTC GCC GGT CAG      480
Glu Arg Leu Ala Lys Ala Ala Gly Pro Glu Gly Met Val Ala Gly Gln
145                 150                 155                 160
GCA GCC GAT ATG GAA GGA GAG GGG AAA ACG CTG ACG CTT TCG GAG CTC      528
Ala Ala Asp Met Glu Gly Glu Gly Lys Thr Leu Thr Leu Ser Glu Leu
                165                 170                 175
GAA TAC ATT CAT CGG CAT AAA ACC GGG AAA ATG CTG CAA TAC AGC GTG      576
Glu Tyr Ile His Arg His Lys Thr Gly Lys Met Leu Gln Tyr Ser Val
            180                 185                 190
CAC GCC GGC GCC TTG ATC GGC GGC GCT GAT GCC CGG CAA ACG CGG GAG      624
His Ala Gly Ala Leu Ile Gly Gly Ala Asp Ala Arg Gln Thr Arg Glu
        195                 200                 205
```

```
CTT GAC GAA TTC GCC GCC CAT CTA GGC CTT GCC TTT CAA ATT CGC GAT     672
Leu Asp Glu Phe Ala Ala His Leu Gly Leu Ala Phe Gln Ile Arg Asp
    210                 215                     220
GAT ATT CTC GAT ATT GAA GGG GCA GAA GAA AAA ATC GGC AAG CCG GTC     720
Asp Ile Leu Asp Ile Glu Gly Ala Glu Glu Lys Ile Gly Lys Pro Val
225                 230                     235                 240
GGC AGC GAC CAA AGC AAC AAC AAA GCG ACG TAT CCA GCG TTG CTG TCG     768
Gly Ser Asp Gln Ser Asn Asn Lys Ala Thr Tyr Pro Ala Leu Leu Ser
                245                     250                 255
CTT GCC GGC GCG AAG GAA AAG TTG GCG TTC CAT ATC GAG GCG GCG CAG     816
Leu Ala Gly Ala Lys Glu Lys Leu Ala Phe His Ile Glu Ala Ala Gln
            260                 265                     270
CGC CAT TTA CGG AAC GCC GAC GTT GAC GGC GCC GCG CTC GCC TAT ATT     864
Arg His Leu Arg Asn Ala Asp Val Asp Gly Ala Ala Leu Ala Tyr Ile
        275                 280                     285
TGC GAA CTG GTC GCC GCC CGC GAC CAT TAA                             894
Cys Glu Leu Val Ala Ala Arg Asp His ***
    290                 295
```

SEQ ID NO: 3

SEQUENCE LENGTH: 894

SEQUENCE TYPE: Nucleic acid

STRANDNESS: Double

TOPOLOY: Linear

MOLECULAR TYPE:

SOURCE: Bacillus stearothermophilus

CHARACTERISTIC: Mutant (3) of DNA coding for farnesyldiphosphate systhase

SEQUENCE

ATG GCG CAG CTT TCA GTT GAA CAG TTT CTC AAC GAG CAA AAA CAG GCG 48
Met Ala Gln Leu Ser Val Glu Gln Phe Leu Asn Glu Gln Lys Gln Ala
5 10 15

GTG GAA ACA GCG CTC TCC CGT TAT ATA GAG CGC TTA GAA GGG CCG GCG 96
Val Glu Thr Ala Leu Ser Arg Tyr Ile Glu Arg Leu Glu Gly Pro Ala
20 25 30

AAG CTG AAA AAG GCG ATG GCG TAC TCA TTG GAG GCC GGC GGC AAA CGA 144
Lys Leu Lys Lys Ala Met Ala Tyr Ser Leu Glu Ala Gly Gly Lys Arg
35 40 45

ATC CGT CCG TTG CTG CTT CTG TCC ACC GTT CGG GCG CTC GGA AAA GAC 192
Ile Arg Pro Leu Leu Leu Leu Ser Thr Val Arg Ala Leu Gly Lys Asp
50 55 60

CCG GCG GTC GGA TTG CCC GTC GCC TGC GCG ATT GAA ATG ATC CAT ACG 240
Pro Ala Val Gly Leu Pro Val Ala Cys Ala Ile Glu Met Ile His Thr
65 70 75 80

TAC TCT TTG ATC CAT GAT GAT TTG CCG AGC ATG GAC AAC GAT GAT TTG 288
Tyr Ser Leu Ile His Asp Asp Leu Pro Ser Met Asp Asn Asp Asp Leu
85 90 95

CGG CGC GGC AAG CCG ACG AAC CAT AAA GTG TTC GGC GAG GCG ATG GCC 336
Arg Arg Gly Lys Pro Thr Asn His Lys Val Phe Gly Glu Ala Met Ala
100 105 110

ATC TTG GCG GGG GAC GGG TTG TTG ACG TAC GCG TTT CAA TTG ATC ACC 384
Ile Leu Ala Gly Asp Gly Leu Leu Thr Tyr Ala Phe Gln Leu Ile Thr
115 120 125

GAA ATC GAC GAT GAG CGC ATC CCT CCT TCC GTC CGG CTT CGG CTC ATC 432
Glu Ile Asp Asp Glu Arg Ile Pro Pro Ser Val Arg Leu Arg Leu Ile
130 135 140

GAA CGG CTG GCG AAA GCG GCC GGT CCG GAA GGG ATG GCC GCC GGT CAG 480
Glu Arg Leu Ala Lys Ala Ala Gly Pro Glu Gly Met Ala Ala Gly Gln
145 150 155 160

```
GCA GCC GAT ATG GAA GGA GAG GGG AAA ACG CTG ACG CTT TCG GAG CTC      528
Ala Ala Asp Met Glu Gly Glu Gly Lys Thr Leu Thr Leu Ser Glu Leu
                165                 170                 175
GAA TAC ATT CAT CGG TAT AAA ACC GGG AAA ATG CTG CAA TAC AGC GTG      576
Glu Tyr Ile His Arg Tyr Lys Thr Gly Lys Met Leu Gln Tyr Ser Val
            180                 185                 190
CAC GCC GGC GCC TTG ATC GGC GGC GCT GAT GCC CGG CAA ACG CGG GAG      624
His Ala Gly Ala Leu Ile Gly Gly Ala Asp Ala Arg Gln Thr Arg Glu
        195                 200                 205
CTT GAC GAA TTC GCC GCC CAT CTA GGC CTT GCC TTT CAA ATT CGC GAT      672
Leu Asp Glu Phe Ala Ala His Leu Gly Leu Ala Phe Gln Ile Arg Asp
    210                 215                 220
GAT ATT CTC GAT ATT GAA GGG GCA GAA GAA AAA ATC GGC AAG CCG GTC      720
Asp Ile Leu Asp Ile Glu Gly Ala Glu Glu Lys Ile Gly Lys Pro Val
225                 230                 235                 240
GGC AGC GAC CAA AGC AAC AAC AAA GCG ACG TAT CCA GCG TTG CTG TCG      768
Gly Ser Asp Gln Ser Asn Asn Lys Ala Thr Tyr Pro Ala Leu Leu Ser
                245                 250                 255
CTT GCC GGC GCA AAG GAA AAG TTG GCG TTC CAT ATC GAG GCG GCG CAG      816
Leu Ala Gly Ala Lys Glu Lys Leu Ala Phe His Ile Glu Ala Ala Gln
            260                 265                 270
CGC CAT TTA CGG AAC GCC GAC GTT GAC GGC GCC GCG CTC GCC TAT ATT      864
Arg His Leu Arg Asn Ala Asp Val Asp Gly Ala Ala Leu Ala Tyr Ile
        275                 280                 285
TGC GAA CTG GTC GCC GCC CGC GAC CAT TAA                              894
Cys Glu Leu Val Ala Ala Arg Asp His ***
    290                 295
```

SEQ ID NO: 4

SEQUENCE LENGTH: 894

SEQUENCE TYPE: Nucleic acid

STRANDNESS: Double

TOPOLOY: Linear

MOLECULAR TYPE:

SOURCE: Bacillus stearothermophilus

CHARACTERISTIC: Mutant (4) of DNA coding for farnesyldiphosphate systhase

SEQUENCE

```
ATG GCG CAG CTT TCA GTT GAA CAG TTT CTC AAC GAG CAA AAA CAG GCG    48
Met Ala Gln Leu Ser Val Glu Gln Phe Leu Asn Glu Gln Lys Gln Ala
                  5                  10                  15
GTG GAA ACA GCG CTC TCC CGT TAT ATA GAG CGC TTA GAA GGG CCG GCG    96
Val Glu Thr Ala Leu Ser Arg Tyr Ile Glu Arg Leu Glu Gly Pro Ala
             20                  25                  30
AAG CTG AAA AAG GCG ATG GCG TAC TCA TTG GAG GCC GGC GGC AAA CGA   144
Lys Leu Lys Lys Ala Met Ala Tyr Ser Leu Glu Ala Gly Gly Lys Arg
         35                  40                  45
ATC CGT CCG TTG CTG CTT CTG TCC ACC GTT CGG GCG CTC GGC AAA GAC   192
Ile Arg Pro Leu Leu Leu Leu Ser Thr Val Arg Ala Leu Gly Lys Asp
     50                  55                  60
CCG GCG GTC GGA TTG CCC GTC GCC TGC GCG ATT GAA ATG ATC CAT ACG   240
Pro Ala Val Gly Leu Pro Val Ala Cys Ala Ile Glu Met Ile His Thr
 65                  70                  75                  80
CAC TCT TTG ATC CAT GAT GAT TTG CCG AGC ATG GAC AAC GAT GAT TTG   288
His Ser Leu Ile His Asp Asp Leu Pro Ser Met Asp Asn Asp Asp Leu
                 85                  90                  95
CGG CGC GGC AAG CCG ACG AAC CAT AAA GTG TTC GGC GAG GCG ATG GCC   336
Arg Arg Gly Lys Pro Thr Asn His Lys Val Phe Gly Glu Ala Met Ala
            100                 105                 110
ATC TTG GCG GGG GAC GGG TTG TTG ACG TAC GCG TTT CAA TTG ATC ACC   384
Ile Leu Ala Gly Asp Gly Leu Leu Thr Tyr Ala Phe Gln Leu Ile Thr
        115                 120                 125
GAA ATC GAC GAT GAG CGC ATC CCT CCT TCC GTC CGG CTT CGG CTC ATC   432
Glu Ile Asp Asp Glu Arg Ile Pro Pro Ser Val Arg Leu Arg Leu Ile
    130                 135                 140
```

GAA CGG CTG GCG AAA GCG GCC GGT CCG GAA GGG ATG GTC GCC GGT CAG 480
Glu Arg Leu Ala Lys Ala Ala Gly Pro Glu Gly Met Val Ala Gly Gln
145 150 155 160
GCA GCC GAT ATG GAA GGA GAG GGG AAA ACG CTG ACG CTT TCG GAG CTC 528
Ala Ala Asp Met Glu Gly Glu Gly Lys Thr Leu Thr Leu Ser Glu Leu
165 170 175
GAA TAC ATT CAT CGG CAT AAA ACC GGG AAA ATG CTG CAA TAC AGC GTG 576
Glu Tyr Ile His Arg His Lys Thr Gly Lys Met Leu Gln Tyr Ser Val
180 185 190
CAC GCC GGC GCC TTG ATC GGC GGC GCT GAT GCC CGG CAA ACG CGG GAG 624
His Ala Gly Ala Leu Ile Gly Gly Ala Asp Ala Arg Gln Thr Arg Glu
195 200 205
CTT GAC GAA TTC GCC GCC CAT CTA GGC CTT GCC TTT CAA ATT CGC GAT 672
Leu Asp Glu Phe Ala Ala His Leu Gly Leu Ala Phe Gln Ile Arg Asp
210 215 220
GAT ATT CTC GAT ATT GAA GGG GCA GAA GAA AAA ATC GGC AAG CGG GTC 720
Asp Ile Leu Asp Ile Glu Gly Ala Glu Glu Lys Ile Gly Lys Arg Val
225 230 235 240
GGC AGC GAC CAA AGC AAC AAC AAA GCG ACG TAT CCA GCG TTG CTG TCG 768
Gly Ser Asp Gln Ser Asn Asn Lys Ala Thr Tyr Pro Ala Leu Leu Ser
245 250 255
CTT GCC GGC GCG AAG GAA AAG TTG ACG TTC CAT ATC GAG GCG GCG CAG 816
Leu Ala Gly Ala Lys Glu Lys Leu Thr Phe His Ile Glu Ala Ala Gln
260 265 270
CGC CAT TTA CGG AAC GCC GAC GTT GAC GGC GCC GCG CTC GCC TAT ATT 864
Arg His Leu Arg Asn Ala Asp Val Asp Gly Ala Ala Leu Ala Tyr Ile
275 280 285
TGC GAA CTG GTC GCC GCC CGC GAC CAT TAA 894
Cys Glu Leu Val Ala Ala Arg Asp His ***
290 295

SEQ ID NO: 5

SEQUENCE LENGTH: 894

SEQUENCE TYPE: Nucleic acid

STRANDNESS: Double

TOPOLOY: Linear

MOLECULAR TYPE:

SOURCE: Bacillus stearothermophilus

CHARACTERISTIC: DNA coding for native farnesyldiphosphate synthase

SEQUENCE

```
ATG GCG CAG CTT TCA GTT GAA CAG TTT CTC AAC GAG CAA AAA CAG GCG      48
Met Ala Gln Leu Ser Val Glu Gln Phe Leu Asn Glu Gln Lys Gln Ala
                  5                  10                  15
GTG GAA ACA GCG CTC TCC CGT TAT ATA GAG CGC TTA GAA GGG CCG GCG      96
Val Glu Thr Ala Leu Ser Arg Tyr Ile Glu Arg Leu Glu Gly Pro Ala
              20                  25                  30
AAG CTG AAA AAG GCG ATG GCG TAC TCA TTG GAG GCC GGC GGC AAA CGA     144
Lys Lys Lys Lys Ala Met Ala Tyr Ser Leu Glu Ala Gly Gly Lys Arg
          35                  40                  45
ATC CGT CCG TTG CTG CTT CTG TCC ACC GTT CAG GCG CTC GGC AAA GAC     192
Ile Arg Pro Leu Leu Leu Leu Ser Thr Val Gln Ala Leu Gly Lys Asp
      50                  55                  60
CCG GCG GTC GGA TTG CCC GTC GCC TGC GCG ATT GAA ATG ATC CAT ACG     240
Pro Ala Val Gly Leu Pro Val Ala Cys Ala Ile Glu Met Ile His Thr
 65                  70                  75                  80
TAC TCT TTG ATC CAT GAT GAT TTG CCG AGC ATG GAC AAC GAT GAT TTG     288
Tyr Ser Leu Ile His Asp Asp Leu Pro Ser Met Asp Asn Asp Asp Leu
                 85                  90                  95
CGG CGC GGC AAG CCG ACG AAC CAT AAA GTG TTC GGC GAG GCG ATG GCC     336
Arg Arg Gly Lys Pro Thr Asn His Lys Val Phe Gly Glu Ala Met Ala
             100                 105                 110
```

ATC TTG GCG GGG GAC GGG TTG TTG ACG TAC GCG TTT CAA TTG ATC ACC 384
Ile Leu Ala Gly Asp Gly Leu Leu Thr Tyr Ala Phe Gln Leu Ile Thr
115 120 125

GAA ATC GAC GAT GAG CGC ATC CCT CCT TCC GTC CGG CTT CGG CTC ATC 432
Glu Ile Asp Asp Glu Arg Ile Pro Pro Ser Val Arg Leu Arg Leu Ile
130 135 140

GAA CGG CTG GCG AAA GCG GCC GGT CCG GAA GGG ATG GTC GCC GGT CAG 480
Glu Arg Leu Ala Lys Ala Ala Gly Pro Glu Gly Met Val Ala Gly Gln
145 150 155 160

GCA GCC GAT ATG GAA GGA GAG GGG AAA ACG CTG ACG CTT TCG GAG CTC 528
Ala Ala Asp Met Glu Gly Glu Gly Lys Thr Leu Thr Leu Ser Glu Leu
165 170 175

GAA TAC ATT CAT CGG CAT AAA ACC GGG AAA ATG CTG CAA TAC AGC GTG 576
Glu Tyr Ile His Arg His Lys Thr Gly Lys Met Leu Gln Tyr Ser Val
180 185 190

CAC GCC GGC GCC TTG ATC GGC GGC GCT GAT GCC CGG CAA ACG CGG GAG 624
His Ala Gly Ala Leu Ile Gly Gly Ala Asp Ala Arg Gln Thr Arg Glu
195 200 205

CTT GAC GAA TTC GCC GCC CAT CTA GGC CTT GCC TTT CAA ATT CGC GAT 672
Leu Asp Glu Phe Ala Ala His Leu Gly Leu Ala Phe Gln Ile Arg Asp
210 215 220

GAT ATT CTC GAT ATT GAA GGG GCA GAA GAA AAA ATC GGC AAG CCG GTC 720
Asp Ile Leu Asp Ile Glu Gly Ala Glu Glu Lys Ile Gly Lys Pro Val
225 230 235 240

GGC AGC GAC CAA AGC AAC AAC AAA GCG ACG TAT CCA GCG TTG CTG TCG 768
Gly Ser Asp Gln Ser Asn Asn Lys Ala Thr Tyr Pro Ala Leu Leu Ser
245 250 255

CTT GCC GGC GCG AAG GAA AAG TTG GCG TTC CAT ATC GAG GCG GCG CAG 816
Leu Ala Gly Ala Lys Glu Lys Leu Ala Phe His Ile Glu Ala Ala Gln
260 265 270

CGC CAT TTA CGG AAC GCC GAC GTT GAC GGC GCC GCG CTC GCC TAT ATT 864
Arg His Leu Arg Asn Ala Asp Val Asp Gly Ala Ala Leu Ala Tyr Ile
275 280 285

TGC GAA CTG GTC GCC GCC CGC GAC CAT TAA 894
Cys Glu Leu Val Ala Ala Arg Asp His ***
290 295

A mutated farnesyldiphosphate synthase capable of synthesizing geranylgeranyldiphosphate and gene coding for said mutated enzyme, wherein the mutated enzyme is modified from a native farnesyldiphosphate synthase by mutation of a gene coding for a native farnesyldiphosphate synthase.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Toyota Jidosha Kabushiki Kaisha
(B) STREET: 1, Toyota-cho
(C) CITY: Toyota-shi
(D) STATE: Aichi
(E) COUNTRY: Japan
(F) POSTAL CODE (ZIP): None

(ii) TITLE OF INVENTION: MUTATED FARNESYLDIPHOSPHATE SYNTHASE CAPABLE OF SYNTHESIZING GERANYLGERANYLDIPHOSPHATE AND GENE CODING THEREFOR

(iii) NUMBER OF SEQUENCES: 10

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: EP 95115423.6

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 894 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bacillus stearothermophilus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..894
(D) OTHER INFORMATION: /function= "Mutant (1) of DNA coding for farnesyldiphosphate synthase"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
ATG GCG CAG CTT TCA GTT GAA CAG TTT CTC AAC GAG CAA AAA CAG GCG        48
Met Ala Gln Leu Ser Val Glu Gln Phe Leu Asn Glu Gln Lys Gln Ala
  1               5                  10                  15

GTG GAA ACA GCG CTC TCC CGT TAT ATA GAG CGC TTA GAA GGG CCG GCG        96
Val Glu Thr Ala Leu Ser Arg Tyr Ile Glu Arg Leu Glu Gly Pro Ala
             20                  25                  30
```

```
AAG CTG AAA AAG GCG ATG GCG TAC TCA TTG GAG GCC GGC GGC AAA CGA      144
Lys Leu Lys Lys Ala Met Ala Tyr Ser Leu Glu Ala Gly Gly Lys Arg
        35                  40                  45

ATC CGT CCG TTG CTG CTT CTG TCC ACC GTT CGG GCG CTC GGA AAA GAC      192
Ile Arg Pro Leu Leu Leu Leu Ser Thr Val Arg Ala Leu Gly Lys Asp
    50                  55                  60

CCG GCG GTC GGA TTG CCC GTC GCC TGC GCG ATT GAA ATG ATC CAT ACG      240
Pro Ala Val Gly Leu Pro Val Ala Cys Ala Ile Glu Met Ile His Thr
65                  70                  75                  80

CAC TCT TTG ATC CAT GAT GAT TTG CCG AGC ATG GAC AAC GAT GAT TTG      288
His Ser Leu Ile His Asp Asp Leu Pro Ser Met Asp Asn Asp Asp Leu
                85                  90                  95

CGG CGC GGC AAG CCG ACG AAC CAT AAA GTG TTC GGC GAG GCG ATG GCC      336
Arg Arg Gly Lys Pro Thr Asn His Lys Val Phe Gly Glu Ala Met Ala
            100                 105                 110

ATC TTG GCG GGG GAC GGG TTG TTG ACG TAC GCG TTT CAA TTG ATC ACC      384
Ile Leu Ala Gly Asp Gly Leu Leu Thr Tyr Ala Phe Gln Leu Ile Thr
        115                 120                 125

GAA ATC GAC GAT GAG CGC ATC CCT CCT TCC GTC CGG CTT CGG CTC ATC      432
Glu Ile Asp Asp Glu Arg Ile Pro Pro Ser Val Arg Leu Arg Leu Ile
    130                 135                 140

GAA CGG CTG GCG AAA GCG GCC GGT CCG GAA GGG ATG GTC GCC GGT CAG      480
Glu Arg Leu Ala Lys Ala Ala Gly Pro Glu Gly Met Val Ala Gly Gln
145                 150                 155                 160

GCA GCC GAT ATG GAA GGA GAG GGG AAA ACG CTG ACG CTT TCG GAG CTC      528
Ala Ala Asp Met Glu Gly Glu Gly Lys Thr Leu Thr Leu Ser Glu Leu
                165                 170                 175

GAA TAC ATT CAT CGG CAT AAA ACC GGG AAA ATG CTG CAA TAC AGC GTG      576
Glu Tyr Ile His Arg His Lys Thr Gly Lys Met Leu Gln Tyr Ser Val
            180                 185                 190

CAC GCC GGC GCC TTG ATC GGC GGC GCT GAT GCC CGG CAA ACG CGG GAG      624
His Ala Gly Ala Leu Ile Gly Gly Ala Asp Ala Arg Gln Thr Arg Glu
        195                 200                 205

CTT GAC GAA TTC GCC GCC CAT CTA GGC CTT GCC TTT CAA ATT CGC GAT      672
Leu Asp Glu Phe Ala Ala His Leu Gly Leu Ala Phe Gln Ile Arg Asp
    210                 215                 220

GAT ATT CTC GAT ATT GAA GGG GCA GAA GAA AAA ATC GGC AAG CCG GTC      720
Asp Ile Leu Asp Ile Glu Gly Ala Glu Glu Lys Ile Gly Lys Pro Val
225                 230                 235                 240

GGC AGC GAC CAA AGC AAC AAC AAA GCG ACG TAT CCA GCG TTG CTG TCG      768
Gly Ser Asp Gln Ser Asn Asn Lys Ala Thr Tyr Pro Ala Leu Leu Ser
                245                 250                 255
```

```
CTT GCC GGC GCG AAG GAA AAG TTG GCG TTC CAT ATC GAG GCG GCG CAG        816
Leu Ala Gly Ala Lys Glu Lys Leu Ala Phe His Ile Glu Ala Ala Gln
            260                 265                 270

CGC CAT TCA CGG AAC GCC GAC GTT GAC GGC GCC GCG CTC GCC TAT ATT        864
Arg His Ser Arg Asn Ala Asp Val Asp Gly Ala Ala Leu Ala Tyr Ile
        275                 280                 285

TGC GAA CTG GTC GCC GCC CGC GAC CAT TAA                                894
Cys Glu Leu Val Ala Ala Arg Asp His
    290                 295
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 297 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Ala Gln Leu Ser Val Glu Gln Phe Leu Asn Glu Gln Lys Gln Ala
 1               5                  10                  15

Val Glu Thr Ala Leu Ser Arg Tyr Ile Glu Arg Leu Glu Gly Pro Ala
            20                  25                  30

Lys Leu Lys Lys Ala Met Ala Tyr Ser Leu Glu Ala Gly Gly Lys Arg
        35                  40                  45

Ile Arg Pro Leu Leu Leu Leu Ser Thr Val Arg Ala Leu Gly Lys Asp
    50                  55                  60

Pro Ala Val Gly Leu Pro Val Ala Cys Ala Ile Glu Met Ile His Thr
65                  70                  75                  80

His Ser Leu Ile His Asp Asp Leu Pro Ser Met Asp Asn Asp Asp Leu
                85                  90                  95

Arg Arg Gly Lys Pro Thr Asn His Lys Val Phe Gly Glu Ala Met Ala
            100                 105                 110

Ile Leu Ala Gly Asp Gly Leu Leu Thr Tyr Ala Phe Gln Leu Ile Thr
        115                 120                 125

Glu Ile Asp Asp Glu Arg Ile Pro Pro Ser Val Arg Leu Arg Leu Ile
    130                 135                 140

Glu Arg Leu Ala Lys Ala Ala Gly Pro Glu Gly Met Val Ala Gly Gln
145                 150                 155                 160

Ala Ala Asp Met Glu Gly Glu Gly Lys Thr Leu Thr Leu Ser Glu Leu
                165                 170                 175

Glu Tyr Ile His Arg His Lys Thr Gly Lys Met Leu Gln Tyr Ser Val
            180                 185                 190

His Ala Gly Ala Leu Ile Gly Gly Ala Asp Ala Arg Gln Thr Arg Glu
        195                 200                 205

Leu Asp Glu Phe Ala Ala His Leu Gly Leu Ala Phe Gln Ile Arg Asp
    210                 215                 220

Asp Ile Leu Asp Ile Glu Gly Ala Glu Glu Lys Ile Gly Lys Pro Val
225                 230                 235                 240

Gly Ser Asp Gln Ser Asn Asn Lys Ala Thr Tyr Pro Ala Leu Leu Ser
                245                 250                 255
```

```
Leu Ala Gly Ala Lys Glu Lys Leu Ala Phe His Ile Glu Ala Ala Gln
            260                 265                 270

Arg His Ser Arg Asn Ala Asp Val Asp Gly Ala Ala Leu Ala Tyr Ile
        275                 280                 285

Cys Glu Leu Val Ala Ala Arg Asp His
    290                 295
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 894 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bacillus stearothermophilus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..894
(D) OTHER INFORMATION: /function= "Mutant (2) of DNA coding for farnesyldiphosphate synthase"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
ATG GCG CAG CTT TCA GTT GAA CAG TTT CTC AAC GAG CAA AAA CAG GCG        48
Met Ala Gln Leu Ser Val Glu Gln Phe Leu Asn Glu Gln Lys Gln Ala
  1               5                  10                  15

GTG GAA ACA GCG CTC TCC CGT TAT ATA GAG CGC TTA GAA GGG CCG GCG        96
Val Glu Thr Ala Leu Ser Arg Tyr Ile Glu Arg Leu Glu Gly Pro Ala
             20                  25                  30

AAG GTG AAA AAG GCG ATG GCG TAC TCA TTG GAG GCC GGC GGC AAA CGA       144
Lys Val Lys Lys Ala Met Ala Tyr Ser Leu Glu Ala Gly Gly Lys Arg
         35                  40                  45

ATC CGT CCG TTG CTG CTT CTG TCC ACC GTT CAG GCG CTC GGC AAA GAC       192
Ile Arg Pro Leu Leu Leu Leu Ser Thr Val Gln Ala Leu Gly Lys Asp
     50                  55                  60

CCG GCG GTC GGA TTG CCC GTC GCC TGC GCG ATT GAA ATG ATC CAT ACG       240
Pro Ala Val Gly Leu Pro Val Ala Cys Ala Ile Glu Met Ile His Thr
 65                  70                  75                  80

TAC TCT TTG ATC CAT GAT GAT TTG CCG AGC ATG GAC AAC GAT GAT TTG       288
Tyr Ser Leu Ile His Asp Asp Leu Pro Ser Met Asp Asn Asp Asp Leu
                 85                  90                  95

CGG CGC GGC AAG CCG ACG AAC CAT AAA GTG TTC GGC GAG GCG ATG GCC       336
Arg Arg Gly Lys Pro Thr Asn His Lys Val Phe Gly Glu Ala Met Ala
            100                 105                 110

ATC TTG GCG GGG GAC GGG TTG TTG ACG TAC GCG TTT CAA TTG ATC ACC       384
Ile Leu Ala Gly Asp Gly Leu Leu Thr Tyr Ala Phe Gln Leu Ile Thr
        115                 120                 125

GAA ATC GAC GAT GAG CGC ATC CCT CCT TCC GTC CGG CTT CGG CTC ATC       432
Glu Ile Asp Asp Glu Arg Ile Pro Pro Ser Val Arg Leu Arg Leu Ile
    130                 135                 140
```

```
GAA CGG CTG GCG AAA GCG GCC GGT CCG GAA GGG ATG GTC GCC GGT CAG      480
Glu Arg Leu Ala Lys Ala Ala Gly Pro Glu Gly Met Val Ala Gly Gln
145                 150                 155                 160

GCA GCC GAT ATG GAA GGA GAG GGG AAA ACG CTG ACG CTT TCG GAG CTC      528
Ala Ala Asp Met Glu Gly Glu Gly Lys Thr Leu Thr Leu Ser Glu Leu
                165                 170                 175

GAA TAC ATT CAT CGG CAT AAA ACC GGG AAA ATG CTG CAA TAC AGC GTG      576
Glu Tyr Ile His Arg His Lys Thr Gly Lys Met Leu Gln Tyr Ser Val
            180                 185                 190

CAC GCC GGC GCC TTG ATC GGC GGC GCT GAT GCC CGG CAA ACG CGG GAG      624
His Ala Gly Ala Leu Ile Gly Gly Ala Asp Ala Arg Gln Thr Arg Glu
        195                 200                 205

CTT GAC GAA TTC GCC GCC CAT CTA GGC CTT GCC TTT CAA ATT CGC GAT      672
Leu Asp Glu Phe Ala Ala His Leu Gly Leu Ala Phe Gln Ile Arg Asp
    210                 215                 220

GAT ATT CTC GAT ATT GAA GGG GCA GAA GAA AAA ATC GGC AAG CCG GTC      720
Asp Ile Leu Asp Ile Glu Gly Ala Glu Glu Lys Ile Gly Lys Pro Val
225                 230                 235                 240

GGC AGC GAC CAA AGC AAC AAC AAA GCG ACG TAT CCA GCG TTG CTG TCG      768
Gly Ser Asp Gln Ser Asn Asn Lys Ala Thr Tyr Pro Ala Leu Leu Ser
                245                 250                 255

CTT GCC GGC GCG AAG GAA AAG TTG GCG TTC CAT ATC GAG GCG GCG CAG      816
Leu Ala Gly Ala Lys Glu Lys Leu Ala Phe His Ile Glu Ala Ala Gln
            260                 265                 270

CGC CAT TTA CGG AAC GCC GAC GTT GAC GGC GCC GCG CTC GCC TAT ATT      864
Arg His Leu Arg Asn Ala Asp Val Asp Gly Ala Ala Leu Ala Tyr Ile
        275                 280                 285

TGC GAA CTG GTC GCC GCC CGC GAC CAT TAA                              894
Cys Glu Leu Val Ala Ala Arg Asp His
    290                 295
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 297 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Met Ala Gln Leu Ser Val Glu Gln Phe Leu Asn Glu Gln Lys Gln Ala
 1               5                  10                  15

Val Glu Thr Ala Leu Ser Arg Tyr Ile Glu Arg Leu Glu Gly Pro Ala
            20                  25                  30

Lys Val Lys Lys Ala Met Ala Tyr Ser Leu Glu Ala Gly Gly Lys Arg
        35                  40                  45

Ile Arg Pro Leu Leu Leu Leu Ser Thr Val Gln Ala Leu Gly Lys Asp
    50                  55                  60

Pro Ala Val Gly Leu Pro Val Ala Cys Ala Ile Glu Met Ile His Thr
65                  70                  75                  80

Tyr Ser Leu Ile His Asp Asp Leu Pro Ser Met Asp Asn Asp Asp Leu
                85                  90                  95

Arg Arg Gly Lys Pro Thr Asn His Lys Val Phe Gly Glu Ala Met Ala
            100                 105                 110

Ile Leu Ala Gly Asp Gly Leu Leu Thr Tyr Ala Phe Gln Leu Ile Thr
        115                 120                 125

Glu Ile Asp Asp Glu Arg Ile Pro Pro Ser Val Arg Leu Arg Leu Ile
    130                 135                 140

Glu Arg Leu Ala Lys Ala Ala Gly Pro Glu Gly Met Val Ala Gly Gln
145                 150                 155                 160

Ala Ala Asp Met Glu Gly Glu Gly Lys Thr Leu Thr Leu Ser Glu Leu
                165                 170                 175

Glu Tyr Ile His Arg His Lys Thr Gly Lys Met Leu Gln Tyr Ser Val
            180                 185                 190

His Ala Gly Ala Leu Ile Gly Gly Ala Asp Ala Arg Gln Thr Arg Glu
        195                 200                 205

Leu Asp Glu Phe Ala Ala His Leu Gly Leu Ala Phe Gln Ile Arg Asp
    210                 215                 220

Asp Ile Leu Asp Ile Glu Gly Ala Glu Glu Lys Ile Gly Lys Pro Val
225                 230                 235                 240

Gly Ser Asp Gln Ser Asn Asn Lys Ala Thr Tyr Pro Ala Leu Leu Ser
                245                 250                 255
```

```
Leu Ala Gly Ala Lys Glu Lys Leu Ala Phe His Ile Glu Ala Ala Gln
            260                 265                 270

Arg His Leu Arg Asn Ala Asp Val Asp Gly Ala Ala Leu Ala Tyr Ile
        275                 280                 285

Cys Glu Leu Val Ala Ala Arg Asp His
    290                 295
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 894 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bacillus stearothermophilus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..894
(D) OTHER INFORMATION: /function= "Mutant (3) of DNA coding for farnesyldiphosphate synthase"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
ATG GCG CAG CTT TCA GTT GAA CAG TTT CTC AAC GAG CAA AAA CAG GCG      48
Met Ala Gln Leu Ser Val Glu Gln Phe Leu Asn Glu Gln Lys Gln Ala
  1               5                  10                  15

GTG GAA ACA GCG CTC TCC CGT TAT ATA GAG CGC TTA GAA GGG CCG GCG      96
Val Glu Thr Ala Leu Ser Arg Tyr Ile Glu Arg Leu Glu Gly Pro Ala
             20                  25                  30

AAG CTG AAA AAG GCG ATG GCG TAC TCA TTG GAG GCC GGC GGC AAA CGA     144
Lys Leu Lys Lys Ala Met Ala Tyr Ser Leu Glu Ala Gly Gly Lys Arg
         35                  40                  45

ATC CGT CCG TTG CTG CTT CTG TCC ACC GTT CGG GCG CTC GGA AAA GAC     192
Ile Arg Pro Leu Leu Leu Leu Ser Thr Val Arg Ala Leu Gly Lys Asp
     50                  55                  60

CCG GCG GTC GGA TTG CCC GTC GCC TGC GCG ATT GAA ATG ATC CAT ACG     240
Pro Ala Val Gly Leu Pro Val Ala Cys Ala Ile Glu Met Ile His Thr
 65                  70                  75                  80

TAC TCT TTG ATC CAT GAT GAT TTG CCG AGC ATG GAC AAC GAT GAT TTG     288
Tyr Ser Leu Ile His Asp Asp Leu Pro Ser Met Asp Asn Asp Asp Leu
                 85                  90                  95

CGG CGC GGC AAG CCG ACG AAC CAT AAA GTG TTC GGC GAG GCG ATG GCC     336
Arg Arg Gly Lys Pro Thr Asn His Lys Val Phe Gly Glu Ala Met Ala
            100                 105                 110

ATC TTG GCG GGG GAC GGG TTG TTG ACG TAC GCG TTT CAA TTG ATC ACC     384
Ile Leu Ala Gly Asp Gly Leu Leu Thr Tyr Ala Phe Gln Leu Ile Thr
        115                 120                 125

GAA ATC GAC GAT GAG CGC ATC CCT CCT TCC GTC CGG CTT CGG CTC ATC     432
Glu Ile Asp Asp Glu Arg Ile Pro Pro Ser Val Arg Leu Arg Leu Ile
    130                 135                 140
```

```
GAA CGG CTG GCG AAA GCG GCC GGT CCG GAA GGG ATG GCC GCC GGT CAG     480
Glu Arg Leu Ala Lys Ala Ala Gly Pro Glu Gly Met Ala Ala Gly Gln
145                 150                 155                 160

GCA GCC GAT ATG GAA GGA GAG GGG AAA ACG CTG ACG CTT TCG GAG CTC     528
Ala Ala Asp Met Glu Gly Glu Gly Lys Thr Leu Thr Leu Ser Glu Leu
                165                 170                 175

GAA TAC ATT CAT CGG TAT AAA ACC GGG AAA ATG CTG CAA TAC AGC GTG     576
Glu Tyr Ile His Arg Tyr Lys Thr Gly Lys Met Leu Gln Tyr Ser Val
            180                 185                 190

CAC GCC GGC GCC TTG ATC GGC GGC GCT GAT GCC CGG CAA ACG CGG GAG     624
His Ala Gly Ala Leu Ile Gly Gly Ala Asp Ala Arg Gln Thr Arg Glu
        195                 200                 205

CTT GAC GAA TTC GCC GCC CAT CTA GGC CTT GCC TTT CAA ATT CGC GAT     672
Leu Asp Glu Phe Ala Ala His Leu Gly Leu Ala Phe Gln Ile Arg Asp
    210                 215                 220

GAT ATT CTC GAT ATT GAA GGG GCA GAA GAA AAA ATC GGC AAG CCG GTC     720
Asp Ile Leu Asp Ile Glu Gly Ala Glu Glu Lys Ile Gly Lys Pro Val
225                 230                 235                 240

GGC AGC GAC CAA AGC AAC AAC AAA GCG ACG TAT CCA GCG TTG CTG TCG     768
Gly Ser Asp Gln Ser Asn Asn Lys Ala Thr Tyr Pro Ala Leu Leu Ser
                245                 250                 255

CTT GCC GGC GCA AAG GAA AAG TTG GCG TTC CAT ATC GAG GCG GCG CAG     816
Leu Ala Gly Ala Lys Glu Lys Leu Ala Phe His Ile Glu Ala Ala Gln
            260                 265                 270

CGC CAT TTA CGG AAC GCC GAC GTT GAC GGC GCC GCG CTC GCC TAT ATT     864
Arg His Leu Arg Asn Ala Asp Val Asp Gly Ala Ala Leu Ala Tyr Ile
        275                 280                 285

TGC GAA CTG GTC GCC GCC CGC GAC CAT TAA                             894
Cys Glu Leu Val Ala Ala Arg Asp His
    290                 295
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 297 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Met Ala Gln Leu Ser Val Glu Gln Phe Leu Asn Glu Gln Lys Gln Ala
  1               5                  10                  15

Val Glu Thr Ala Leu Ser Arg Tyr Ile Glu Arg Leu Glu Gly Pro Ala
             20                  25                  30

Lys Leu Lys Lys Ala Met Ala Tyr Ser Leu Glu Ala Gly Gly Lys Arg
         35                  40                  45

Ile Arg Pro Leu Leu Leu Leu Ser Thr Val Arg Ala Leu Gly Lys Asp
     50                  55                  60

Pro Ala Val Gly Leu Pro Val Ala Cys Ala Ile Glu Met Ile His Thr
 65                  70                  75                  80

Tyr Ser Leu Ile His Asp Asp Leu Pro Ser Met Asp Asn Asp Asp Leu
                 85                  90                  95

Arg Arg Gly Lys Pro Thr Asn His Lys Val Phe Gly Glu Ala Met Ala
            100                 105                 110

Ile Leu Ala Gly Asp Gly Leu Leu Thr Tyr Ala Phe Gln Leu Ile Thr
        115                 120                 125

Glu Ile Asp Asp Glu Arg Ile Pro Pro Ser Val Arg Leu Arg Leu Ile
    130                 135                 140

Glu Arg Leu Ala Lys Ala Ala Gly Pro Glu Gly Met Ala Ala Gly Gln
145                 150                 155                 160

Ala Ala Asp Met Glu Gly Glu Gly Lys Thr Leu Thr Leu Ser Glu Leu
                165                 170                 175

Glu Tyr Ile His Arg Tyr Lys Thr Gly Lys Met Leu Gln Tyr Ser Val
            180                 185                 190

His Ala Gly Ala Leu Ile Gly Gly Ala Asp Ala Arg Gln Thr Arg Glu
        195                 200                 205

Leu Asp Glu Phe Ala Ala His Leu Gly Leu Ala Phe Gln Ile Arg Asp
    210                 215                 220

Asp Ile Leu Asp Ile Glu Gly Ala Glu Glu Lys Ile Gly Lys Pro Val
225                 230                 235                 240

Gly Ser Asp Gln Ser Asn Asn Lys Ala Thr Tyr Pro Ala Leu Leu Ser
                245                 250                 255
```

```
Leu Ala Gly Ala Lys Glu Lys Leu Ala Phe His Ile Glu Ala Ala Gln
            260                 265                     270

Arg His Leu Arg Asn Ala Asp Val Asp Gly Ala Ala Leu Ala Tyr Ile
        275                 280                     285

Cys Glu Leu Val Ala Ala Arg Asp His
    290                 295
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 894 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bacillus stearothermophilus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..894
(D) OTHER INFORMATION: /function= "Mutant (4) of DNA coding for farnesyldiphosphate synthase"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
ATG GCG CAG CTT TCA GTT GAA CAG TTT CTC AAC GAG CAA AAA CAG GCG       48
Met Ala Gln Leu Ser Val Glu Gln Phe Leu Asn Glu Gln Lys Gln Ala
  1               5                  10                  15

GTG GAA ACA GCG CTC TCC CGT TAT ATA GAG CGC TTA GAA GGG CCG GCG       96
Val Glu Thr Ala Leu Ser Arg Tyr Ile Glu Arg Leu Glu Gly Pro Ala
             20                  25                  30

AAG CTG AAA AAG GCG ATG GCG TAC TCA TTG GAG GCC GGC GGC AAA CGA      144
Lys Leu Lys Lys Ala Met Ala Tyr Ser Leu Glu Ala Gly Gly Lys Arg
         35                  40                  45

ATC CGT CCG TTG CTG CTT CTG TCC ACC GTT CGG GCG CTC GGC AAA GAC      192
Ile Arg Pro Leu Leu Leu Leu Ser Thr Val Arg Ala Leu Gly Lys Asp
     50                  55                  60

CCG GCG GTC GGA TTG CCC GTC GCC TGC GCG ATT GAA ATG ATC CAT ACG      240
Pro Ala Val Gly Leu Pro Val Ala Cys Ala Ile Glu Met Ile His Thr
 65                  70                  75                  80

CAC TCT TTG ATC CAT GAT GAT TTG CCG AGC ATG GAC AAC GAT GAT TTG      288
His Ser Leu Ile His Asp Asp Leu Pro Ser Met Asp Asn Asp Asp Leu
                 85                  90                  95

CGG CGC GGC AAG CCG ACG AAC CAT AAA GTG TTC GGC GAG GCG ATG GCC      336
Arg Arg Gly Lys Pro Thr Asn His Lys Val Phe Gly Glu Ala Met Ala
            100                 105                 110

ATC TTG GCG GGG GAC GGG TTG TTG ACG TAC GCG TTT CAA TTG ATC ACC      384
Ile Leu Ala Gly Asp Gly Leu Leu Thr Tyr Ala Phe Gln Leu Ile Thr
        115                 120                 125

GAA ATC GAC GAT GAG CGC ATC CCT CCT TCC GTC CGG CTT CGG CTC ATC      432
Glu Ile Asp Asp Glu Arg Ile Pro Pro Ser Val Arg Leu Arg Leu Ile
    130                 135                 140
```

```
GAA CGG CTG GCG AAA GCG GCC GGT CCG GAA GGG ATG GTC GCC GGT CAG      480
Glu Arg Leu Ala Lys Ala Ala Gly Pro Glu Gly Met Val Ala Gly Gln
145                 150                 155                 160

GCA GCC GAT ATG GAA GGA GAG GGG AAA ACG CTG ACG CTT TCG GAG CTC      528
Ala Ala Asp Met Glu Gly Glu Gly Lys Thr Leu Thr Leu Ser Glu Leu
                165                 170                 175

GAA TAC ATT CAT CGG CAT AAA ACC GGG AAA ATG CTG CAA TAC AGC GTG      576
Glu Tyr Ile His Arg His Lys Thr Gly Lys Met Leu Gln Tyr Ser Val
            180                 185                 190

CAC GCC GGC GCC TTG ATC GGC GGC GCT GAT GCC CGG CAA ACG CGG GAG      624
His Ala Gly Ala Leu Ile Gly Gly Ala Asp Ala Arg Gln Thr Arg Glu
        195                 200                 205

CTT GAC GAA TTC GCC GCC CAT CTA GGC CTT GCC TTT CAA ATT CGC GAT      672
Leu Asp Glu Phe Ala Ala His Leu Gly Leu Ala Phe Gln Ile Arg Asp
    210                 215                 220

GAT ATT CTC GAT ATT GAA GGG GCA GAA GAA AAA ATC GGC AAG CGG GTC      720
Asp Ile Leu Asp Ile Glu Gly Ala Glu Glu Lys Ile Gly Lys Arg Val
225                 230                 235                 240

GGC AGC GAC CAA AGC AAC AAC AAA GCG ACG TAT CCA GCG TTG CTG TCG      768
Gly Ser Asp Gln Ser Asn Asn Lys Ala Thr Tyr Pro Ala Leu Leu Ser
                245                 250                 255

CTT GCC GGC GCG AAG GAA AAG TTG ACG TTC CAT ATC GAG GCG GCG CAG      816
Leu Ala Gly Ala Lys Glu Lys Leu Thr Phe His Ile Glu Ala Ala Gln
            260                 265                 270

CGC CAT TTA CGG AAC GCC GAC GTT GAC GGC GCC GCG CTC GCC TAT ATT      864
Arg His Leu Arg Asn Ala Asp Val Asp Gly Ala Ala Leu Ala Tyr Ile
        275                 280                 285

TGC GAA CTG GTC GCC GCC CGC GAC CAT TAA                              894
Cys Glu Leu Val Ala Ala Arg Asp His
    290                 295
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 297 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Met Ala Gln Leu Ser Val Glu Gln Phe Leu Asn Glu Gln Lys Gln Ala
 1               5                  10                  15

Val Glu Thr Ala Leu Ser Arg Tyr Ile Glu Arg Leu Glu Gly Pro Ala
            20                  25                  30

Lys Leu Lys Lys Ala Met Ala Tyr Ser Leu Glu Ala Gly Gly Lys Arg
        35                  40                  45

Ile Arg Pro Leu Leu Leu Leu Ser Thr Val Arg Ala Leu Gly Lys Asp
    50                  55                  60

Pro Ala Val Gly Leu Pro Val Ala Cys Ala Ile Glu Met Ile His Thr
65                  70                  75                  80

His Ser Leu Ile His Asp Asp Leu Pro Ser Met Asp Asn Asp Asp Leu
                85                  90                  95

Arg Arg Gly Lys Pro Thr Asn His Lys Val Phe Gly Glu Ala Met Ala
            100                 105                 110

Ile Leu Ala Gly Asp Gly Leu Leu Thr Tyr Ala Phe Gln Leu Ile Thr
        115                 120                 125

Glu Ile Asp Asp Glu Arg Ile Pro Pro Ser Val Arg Leu Arg Leu Ile
    130                 135                 140

Glu Arg Leu Ala Lys Ala Ala Gly Pro Glu Gly Met Val Ala Gly Gln
145                 150                 155                 160

Ala Ala Asp Met Glu Gly Glu Gly Lys Thr Leu Thr Leu Ser Glu Leu
                165                 170                 175

Glu Tyr Ile His Arg His Lys Thr Gly Lys Met Leu Gln Tyr Ser Val
            180                 185                 190

His Ala Gly Ala Leu Ile Gly Gly Ala Asp Ala Arg Gln Thr Arg Glu
        195                 200                 205

Leu Asp Glu Phe Ala Ala His Leu Gly Leu Ala Phe Gln Ile Arg Asp
    210                 215                 220

Asp Ile Leu Asp Ile Glu Gly Ala Glu Glu Lys Ile Gly Lys Arg Val
225                 230                 235                 240

Gly Ser Asp Gln Ser Asn Asn Lys Ala Thr Tyr Pro Ala Leu Leu Ser
                245                 250                 255
```

```
Leu Ala Gly Ala Lys Glu Lys Leu Thr Phe His Ile Glu Ala Ala Gln
            260                 265                 270

Arg His Leu Arg Asn Ala Asp Val Asp Gly Ala Ala Leu Ala Tyr Ile
        275                 280                 285

Cys Glu Leu Val Ala Ala Arg Asp His
    290                 295
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 894 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bacillus stearothermophilus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..894
(D) OTHER INFORMATION: /function= "DNA coding for native farnesyldiphosphate synthase"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
ATG GCG CAG CTT TCA GTT GAA CAG TTT CTC AAC GAG CAA AAA CAG GCG      48
Met Ala Gln Leu Ser Val Glu Gln Phe Leu Asn Glu Gln Lys Gln Ala
  1               5                  10                  15

GTG GAA ACA GCG CTC TCC CGT TAT ATA GAG CGC TTA GAA GGG CCG GCG      96
Val Glu Thr Ala Leu Ser Arg Tyr Ile Glu Arg Leu Glu Gly Pro Ala
             20                  25                  30

AAG CTG AAA AAG GCG ATG GCG TAC TCA TTG GAG GCC GGC GGC AAA CGA     144
Lys Lys Lys Lys Ala Met Ala Tyr Ser Leu Glu Ala Gly Gly Lys Arg
         35                  40                  45

ATC CGT CCG TTG CTG CTT CTG TCC ACC GTT CAG GCG CTC GGC AAA GAC     192
Ile Arg Pro Leu Leu Leu Leu Ser Thr Val Gln Ala Leu Gly Lys Asp
     50                  55                  60

CCG GCG GTC GGA TTG CCC GTC GCC TGC GCG ATT GAA ATG ATC CAT ACG     240
Pro Ala Val Gly Leu Pro Val Ala Cys Ala Ile Glu Met Ile His Thr
 65                  70                  75                  80

TAC TCT TTG ATC CAT GAT GAT TTG CCG AGC ATG GAC AAC GAT GAT TTG     288
Tyr Ser Leu Ile His Asp Asp Leu Pro Ser Met Asp Asn Asp Asp Leu
                 85                  90                  95

CGG CGC GGC AAG CCG ACG AAC CAT AAA GTG TTC GGC GAG GCG ATG GCC     336
Arg Arg Gly Lys Pro Thr Asn His Lys Val Phe Gly Glu Ala Met Ala
            100                 105                 110

ATC TTG GCG GGG GAC GGG TTG TTG ACG TAC GCG TTT CAA TTG ATC ACC     384
Ile Leu Ala Gly Asp Gly Leu Leu Thr Tyr Ala Phe Gln Leu Ile Thr
        115                 120                 125

GAA ATC GAC GAT GAG CGC ATC CCT CCT TCC GTC CGG CTT CGG CTC ATC     432
Glu Ile Asp Asp Glu Arg Ile Pro Pro Ser Val Arg Leu Arg Leu Ile
    130                 135                 140
```

```
GAA CGG CTG GCG AAA GCG GCC GGT CCG GAA GGG ATG GTC GCC GGT CAG      480
Glu Arg Leu Ala Lys Ala Ala Gly Pro Glu Gly Met Val Ala Gly Gln
145                 150                 155                 160

GCA GCC GAT ATG GAA GGA GAG GGG AAA ACG CTG ACG CTT TCG GAG CTC      528
Ala Ala Asp Met Glu Gly Glu Gly Lys Thr Leu Thr Leu Ser Glu Leu
                165                 170                 175

GAA TAC ATT CAT CGG CAT AAA ACC GGG AAA ATG CTG CAA TAC AGC GTG      576
Glu Tyr Ile His Arg His Lys Thr Gly Lys Met Leu Gln Tyr Ser Val
            180                 185                 190

CAC GCC GGC GCC TTG ATC GGC GGC GCT GAT GCC CGG CAA ACG CGG GAG      624
His Ala Gly Ala Leu Ile Gly Gly Ala Asp Ala Arg Gln Thr Arg Glu
        195                 200                 205

CTT GAC GAA TTC GCC GCC CAT CTA GGC CTT GCC TTT CAA ATT CGC GAT      672
Leu Asp Glu Phe Ala Ala His Leu Gly Leu Ala Phe Gln Ile Arg Asp
    210                 215                 220

GAT ATT CTC GAT ATT GAA GGG GCA GAA GAA AAA ATC GGC AAG CCG GTC      720
Asp Ile Leu Asp Ile Glu Gly Ala Glu Glu Lys Ile Gly Lys Pro Val
225                 230                 235                 240

GGC AGC GAC CAA AGC AAC AAC AAA GCG ACG TAT CCA GCG TTG CTG TCG      768
Gly Ser Asp Gln Ser Asn Asn Lys Ala Thr Tyr Pro Ala Leu Leu Ser
                245                 250                 255

CTT GCC GGC GCG AAG GAA AAG TTG GCG TTC CAT ATC GAG GCG GCG CAG      816
Leu Ala Gly Ala Lys Glu Lys Leu Ala Phe His Ile Glu Ala Ala Gln
            260                 265                 270

CGC CAT TTA CGG AAC GCC GAC GTT GAC GGC GCC GCG CTC GCC TAT ATT      864
Arg His Leu Arg Asn Ala Asp Val Asp Gly Ala Ala Leu Ala Tyr Ile
        275                 280                 285

TGC GAA CTG GTC GCC GCC CGC GAC CAT TAA                              894
Cys Glu Leu Val Ala Ala Arg Asp His
    290                 295
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 297 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
Met Ala Gln Leu Ser Val Glu Gln Phe Leu Asn Glu Gln Lys Gln Ala
  1               5                  10                  15

Val Glu Thr Ala Leu Ser Arg Tyr Ile Glu Arg Leu Glu Gly Pro Ala
             20                  25                  30

Lys Lys Lys Lys Ala Met Ala Tyr Ser Leu Glu Ala Gly Gly Lys Arg
         35                  40                  45

Ile Arg Pro Leu Leu Leu Leu Ser Thr Val Gln Ala Leu Gly Lys Asp
     50                  55                  60

Pro Ala Val Gly Leu Pro Val Ala Cys Ala Ile Glu Met Ile His Thr
 65                  70                  75                  80

Tyr Ser Leu Ile His Asp Asp Leu Pro Ser Met Asp Asn Asp Asp Leu
                 85                  90                  95

Arg Arg Gly Lys Pro Thr Asn His Lys Val Phe Gly Glu Ala Met Ala
            100                 105                 110

Ile Leu Ala Gly Asp Gly Leu Leu Thr Tyr Ala Phe Gln Leu Ile Thr
        115                 120                 125

Glu Ile Asp Asp Glu Arg Ile Pro Pro Ser Val Arg Leu Arg Leu Ile
    130                 135                 140

Glu Arg Leu Ala Lys Ala Ala Gly Pro Glu Gly Met Val Ala Gly Gln
145                 150                 155                 160

Ala Ala Asp Met Glu Gly Glu Gly Lys Thr Leu Thr Leu Ser Glu Leu
                165                 170                 175

Glu Tyr Ile His Arg His Lys Thr Gly Lys Met Leu Gln Tyr Ser Val
            180                 185                 190

His Ala Gly Ala Leu Ile Gly Gly Ala Asp Ala Arg Gln Thr Arg Glu
        195                 200                 205

Leu Asp Glu Phe Ala Ala His Leu Gly Leu Ala Phe Gln Ile Arg Asp
    210                 215                 220

Asp Ile Leu Asp Ile Glu Gly Ala Glu Glu Lys Ile Gly Lys Pro Val
225                 230                 235                 240

Gly Ser Asp Gln Ser Asn Asn Lys Ala Thr Tyr Pro Ala Leu Leu Ser
                245                 250                 255
```

```
Leu Ala Gly Ala Lys Glu Lys Leu Ala Phe His Ile Glu Ala Ala Gln
            260                 265                 270

Arg His Leu Arg Asn Ala Asp Val Asp Gly Ala Ala Leu Ala Tyr Ile
        275                 280                 285

Cys Glu Leu Val Ala Ala Arg Asp His
    290                 295
```

Claims

1. A process for production of a gene coding for a mutated farnesyldiphosphate synthase capable of synthesizing geranylgeranyldiphosphate synthase comprising the steps of:

   (1) subjecting a gene coding for a farnesyldiphosphate synthase to mutagenesis;
   (2) expressing the genes subjected to the mutagenesis; and
   (3) selecting a gene coding for a mutated farnesyldiphosphate synthase capable of synthesizing geranylgeranyldiphosphate.

2. A process according to claim 1, wherein the gene coding for mutated farnesyldiphosphate synthase capable of synthesizing geranylgeranyldiphosphate is derived from Bacillus stearothermophilus.

3. A gene coding for a mutated farnesyldiphosphate synthase capable of synthesizing geranylgeranyldiphosphate synthase, obtainable according to a process of claim 1.

4. A process for production of a mutated farnesyldiphosphate synthase capable of synthesizing geranylgeranyldiphosphate, comprising the step of expressing a gene of claim 3.

5. A mutated farnesyldiphosphate synthase capable of synthesizing geranylgeranyldiphosphate, obtainable according to a process of claim 4.

6. A process for production of geranylgeranyldiphosphate or geranylgeranyol, comprising the step of acting a mutated farnesyldiphosphate synthase capable of synthesizing geranylgeranyldiphosphate on a substrate selected from the group consisting of isopentenyldiphosphate, dimethylallyldiphosphate, geranyldiphosphate and farnesyldiphosphate.

7. A geranylgeranyldiphosphate synthase having an amino acid sequence modified from a native amino acid sequence of a farnesyldiphosphate synthase wherein the modification comprises deletion of one to a few amino acid residues, addition of one to a few amino acid residues or replacement of one to a few amino acid residues with other amino acid residues, or a combination of said modification.

8. A geranylgeranyldiphosphate synthase according to claim 7, wherein the modification is present on at least one of the positions 34, 59, 81, 157, 182, 239, 265 and 275 of farnesyldiphosphate synthase of Bacillus stearothermophilus origin, or one to a few corresponding positions in an amino acid sequence of a farnesyldiphosphate synthase of other origin.

9. A geranylgeranyldiphosphate synthase having an amino acid sequence shown in SEQ ID NO: 1.

10. A geranylgeranyldiphosphate synthase having an amino acid sequence shown in SEQ ID NO: 2.

11. A geranylgeranyldiphosphate synthase having an amino acid sequence shown in SEQ ID NO: 3.

12. A geranylgeranyldiphosphate synthase having an amino acid sequence shown in SEQ ID NO: 4.

13. A gene coding for a geranylgeranyldiphosphate synthase according to claim 7.

14. A gene coding for a geranylgeranyldiphosphate synthase according to claim 8.

15. A gene coding for a geranylgeranyldiphosphate synthase according to claim 9.

16. A gene coding for a geranylgeranyldiphosphate synthase according to claim 10.

17. A gene coding for a geranylgeranyldiphosphate synthase according to claim 11.

18. A gene coding for a geranylgeranyldiphosphate synthase according to claim 12.

19. An expression vector comprising a gene according to claim 13.

20. An expression vector comprising a gene according to claim 14.

21. An expression vector comprising a gene according to claim 15.

22. An expression vector comprising a gene according to claim 16.

23. An expression vector comprising a gene according to claim 17.

24. An expression vector comprising a gene according to claim 18.

25. A recombinant host transformed with an expression vector according to claim 19.

26. A recombinant host transformed with an expression vector according to claim 20.

27. A recombinant host transformed with an expression vector according to claim 21.

28. A recombinant host transformed with an expression vector according to claim 22.

29. A recombinant host transformed with an expression vector according to claim 23.

30. A recombinant host transformed with an expression vector according to claim 24.

Fig.1

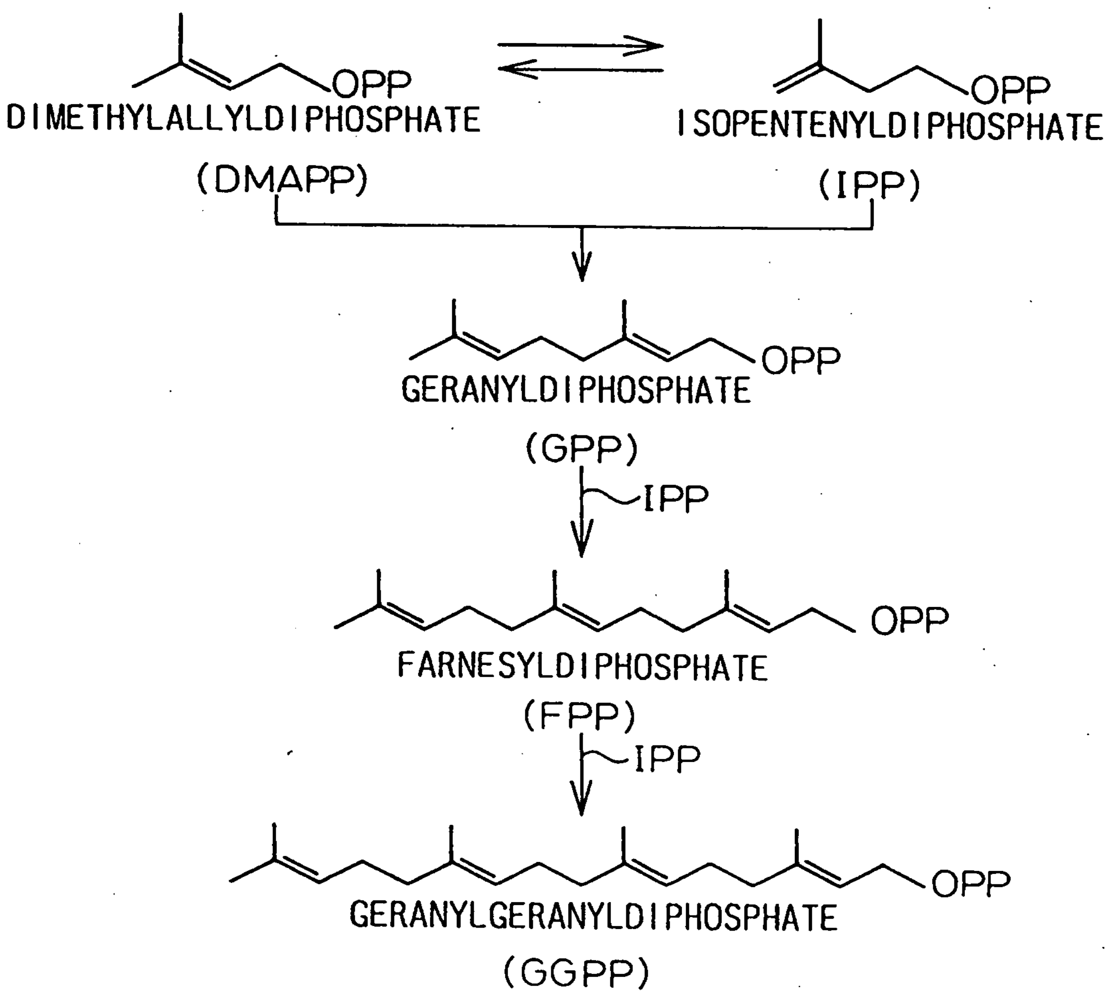

OPP
DIMETHYLALLYLDIPHOSPHATE
(DMAPP)
OPP
ISOPENTENYLDIPHOSPHATE
(IPP)
OPP
GERANYLDIPHOSPHATE
(GPP)
IPP
OPP
FARNESYLDIPHOSPHATE
(FPP)
IPP
OPP
GERANYLGERANYLDIPHOSPHATE
(GGPP)

Fig.2

		A	
(1)	MAQLSVEQFLNEQKQAVETALSRYIERLEGPAKLKKAM	A YSLEAGGKRIRPL	LLLST
(2)	MDFPQQLEACVKQANQALSRFIAPLPFQNTPVVETM	Q YGALLGGKRLRPF	LVYAT
(3)	MASEKEIRRERFLNVFPKLVEELNASLLAYGMPKEACDWYAHS	LNY NTPGGKLNRGL	SVVDT
(4)	MNGDQNSDVYAQEKQDFVQHFSQIVRVLTEDEMGHPEIGDAIARLKEV	LEY NAIGGKYNRGL	TVVVA
(5)	MNGDQKLDVHNQEKQNFIQHFSQIVKVLTEDELGHPEKGDAITRIKEV	LEY NTVGGKYNRGL	TVVQT

		B	
(1)	VRALGKDPAVGLPVA	CAI EMIHT YSLIHDDLPSMDNDDLRRGKPTN	HKVFGEAMAIL
(2)	GHMFGVSTNTLDAPAAAVE	C I H AYSLIHDDLPAMDDDDLRRGLPTC	HVKFGEANAIL
(3)	YAILSNKTVEQLGQEEYEKVAILGW	C I ELLQ AYFLVADD MMDKSITRRGQP C	WYKVPEVGEI
(4)	FRELVEPRKQDADSLQRAWTVGW	C VELLQ AFFLVADD IMDSSLTRRGQ TC	WYQKPGVGLD
(5)	FQELVEPRKQDAESLQRALTVGW	C VELLQ AFFLVLDD IMDSSHTRRGQI C	WYQKPGIGLD

	C		D	
(1)	A GDG LLTYA	FQLITEIDDERIPPSVRLRLIERLAKAAGPEGMVA	GQAADM	EGEGKTLTLSE
(2)	A GDALQTL A	FSILSDADMPEVSDRDRISMISELASASGIAGMCG	GQALDL	DAEGKHVPLDA
(3)	AINDAF ML EA	AIYKLLKSHFRNEKYYIDITELFHEVTFQTEL	GQLMDL	ITAPEDKVDLS
(4)	AINDAN LL EA	CIYRLLKLYCREQPYYLNLIELFLQSSYQTEI	GQTLDL	LTAPQGNVDLV
(5)	AINDAL LL EA	AIYRLLKFYCREQPYYLNLLELFLQSSYQTEI	GQTLDL	ITAPQGQVDLG

Fig.3

```
                                   E
(1)  LEYIHRH         KTGKMLQYSVHAG ALIG G ADAR  QTRELDEFAAHL
(2)  LERIHRH         KTGA LIRAAVRLGALS AG  DKG  RRALPVLDKYAESI
(3)  KFSLKKHSFIVTF   KTAYYSFYLPVAL AMYVAGITDEK  DLKQARDVLIPL
(4)  RFTEKRYKSIVKY   KTAFYSFYLPIAA AMYMAGI D G  EKEHANAKKILLEM
(5)  RYTEKRYKSIVKY   KTAFYSFYLPIAA AMYMAGI D G  EKEHANALKILLEM
```

```
          F
(1)  GLAFQIRDDILDIEGAEEKI GKPVGSD QSNNKAT  YPALLSLAGAKEKLAFHIEAAQRHLRNADVDGAA
(2)  GLAFQVQDDILDVVGDTA TLGKRQGAD QQLGK S  TYPALLGLEQARKKARDLIDDARQSLKQLAEQSLDTS
(3)  GEYFQIQDDYLDCFGTPEQI GKI GTDIQDN KCS  WVINKALELASAEQRKTLDENYGKKDSVAEAKCKKIF
(4)  GEFFQIQDDYLDLFGDPSVT GKI GTDIQDN KCS  WLVVQCLQRATPEQYQILKENYGQKAEKVARVKALYE
(5)  GEFFQIQDDYLDLFGDPSVT GKV GTDIQDN KCS  WLVVQCLLRATPQQRQILEENYGQKDPEKVARVKALY
```

```
                                                 G
(1)                                          L AYICELVAARDH    (1) B.STEAROTHERMOPHILAS
(2)  A                                       LEA LADYIIQRNK    (2) E.COLI
(3)  NDLKIEQLYHEYEESIAKDLKAKISQVDESRGFKADV   LTAFLN KVYKRSK    (3) YEAST
(4)  ELDLPAVFLQYEEDSYSHIMALIEQYAAPLPPAVF     LG  LARKIYKRRK    (4) HUMAN
(5)  EELDLRSVFFKYEEDSYNRLKSLIEQCSAPLPPSIF    LE  LANKIYKRRK    (5) RAT
```

Fig.4

```
              2                               34
W.T      1 : MAQLSVEQFLNEQKQAVETALSRYIERLEGPAKLKKAMAYSLEAGGKRIR
No.1     1 :
No.2     1 :                                  V
No.3     1 :
No.4     1 :

                     59                    81
W.T     51 : PLLLLSTVRALGKDPAVGLPVACAIEMIHTYSLIHDDLPSMDNDDLRRGK
No.1    51 :                               H
No.2    51 :         Q
No.3    51 :
No.4    51 :                               H

                                             141
W.T    101 : PTNHKVFGEAMAILAGDGLLTYAFQLITEIDDERIPPSVRLRLIERLAKA
No.1   101 :
No.2   101 :
No.3   101 :
No.4   101 :

                   157                      182
W.T    151 : AGPEGMVAGQAADMEGEGKTLTLSELEYIHRHKTGKMLQYSVHAGALIGG
No.1   151 :
No.2   151 :
No.3   151 :       A                        Y
No.4   151 :

                                                  239
W.T    201 : ADARQTRELDEFAAHLGLAFQIRDDILDIEGAEEKIGKPVGSDQSNNKAT
No.1   201 :
No.2   201 :
No.3   201 :
No.4   201 :                                       R

                           265       275
W.T    251 : YPALLSLAGAKEKLAFHIEAAQRHLRNADVDGAALAYICELVAARDHX
No.1   251 :                         S
No.2   251 :
No.3   251 :
No.4   251 :               T
```

Fig. 5

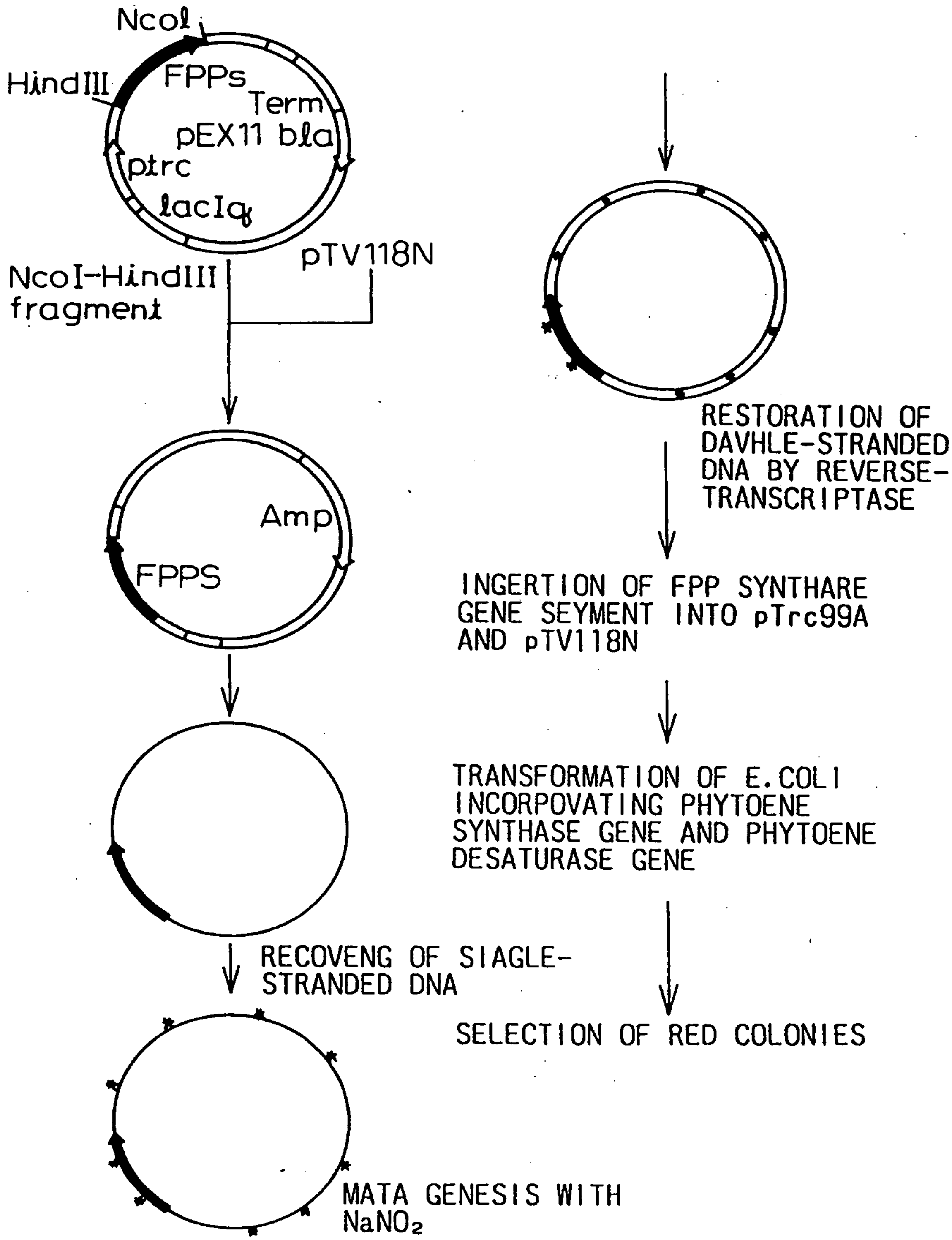

NcoI
HindIII
FPPs
Term
pEX11 bla
ptrc
lacIq
pTV118N
NcoI-HindIII fragment
Amp
FPPS
RESTORATION OF DAVHLE-STRANDED DNA BY REVERSE-TRANSCRIPTASE
INGERTION OF FPP SYNTHARE GENE SEYMENT INTO pTrc99A AND pTV118N
TRANSFORMATION OF E.COLI INCORPOVATING PHYTOENE SYNTHASE GENE AND PHYTOENE DESATURASE GENE
RECOVENG OF SIAGLE-STRANDED DNA
SELECTION OF RED COLONIES
MATA GENESIS WITH $NaNO_2$

Fig.6

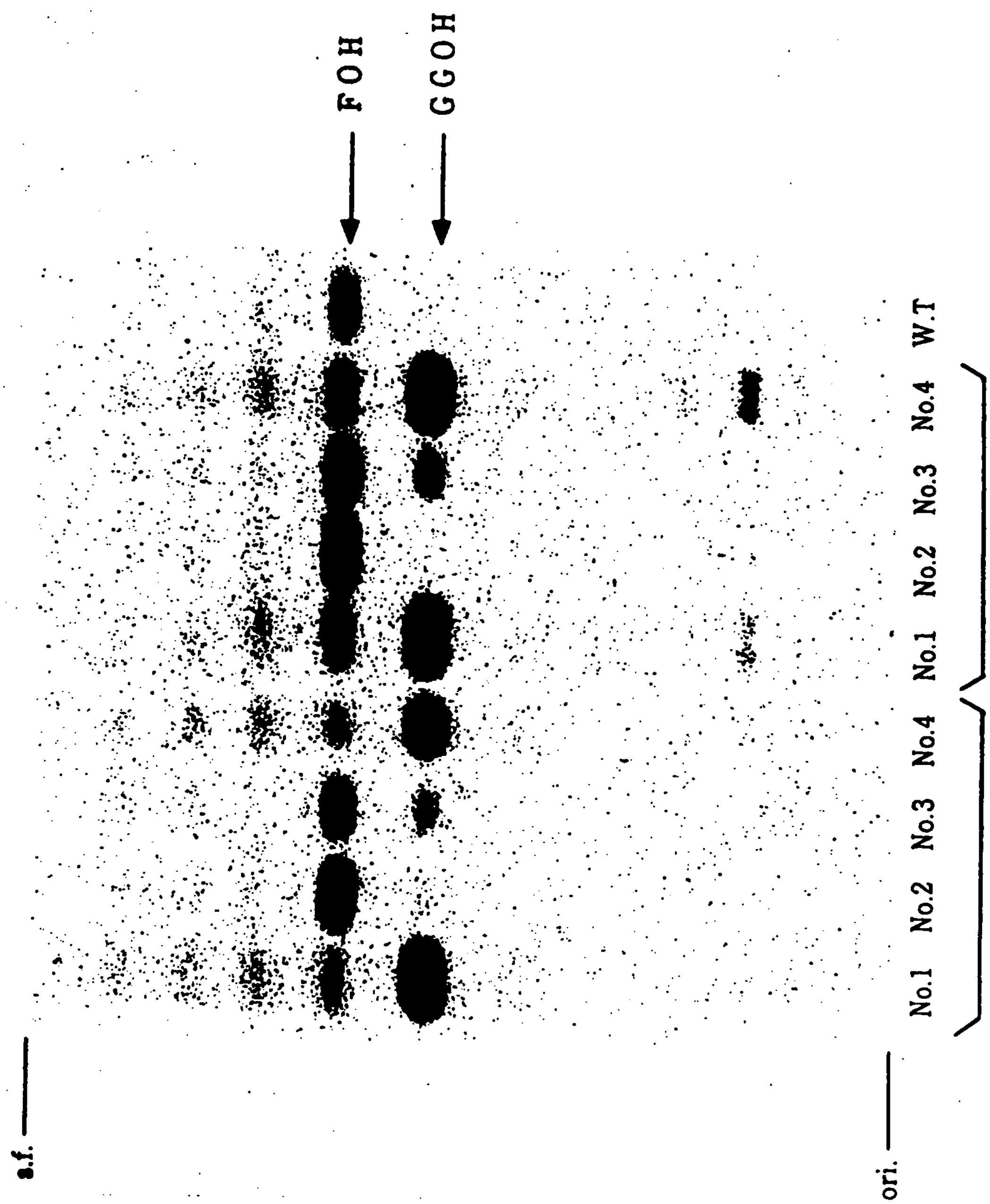

FOH
GGOH
s.f.
ori.
No.1
No.2
No.3
No.4
pTV118N
No.1
No.2
No.3
No.4
pTrc99A
W.T

Fig. 7

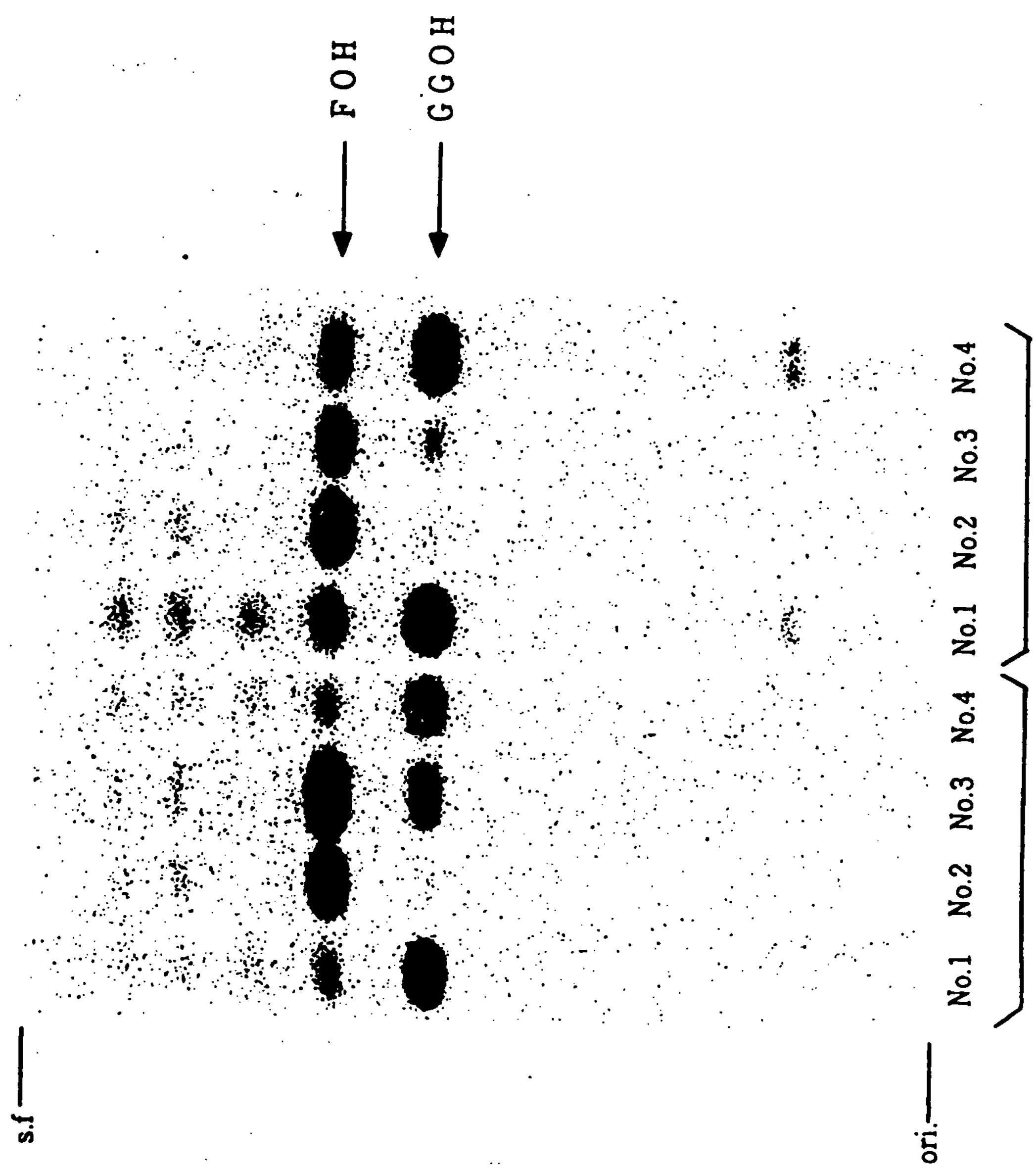

FOH
GGOH
s.f
ori.
No.1
No.2
No.3
No.4
pTV118N
No.1
No.2
No.3
No.4
pTrc99A

Fig.8

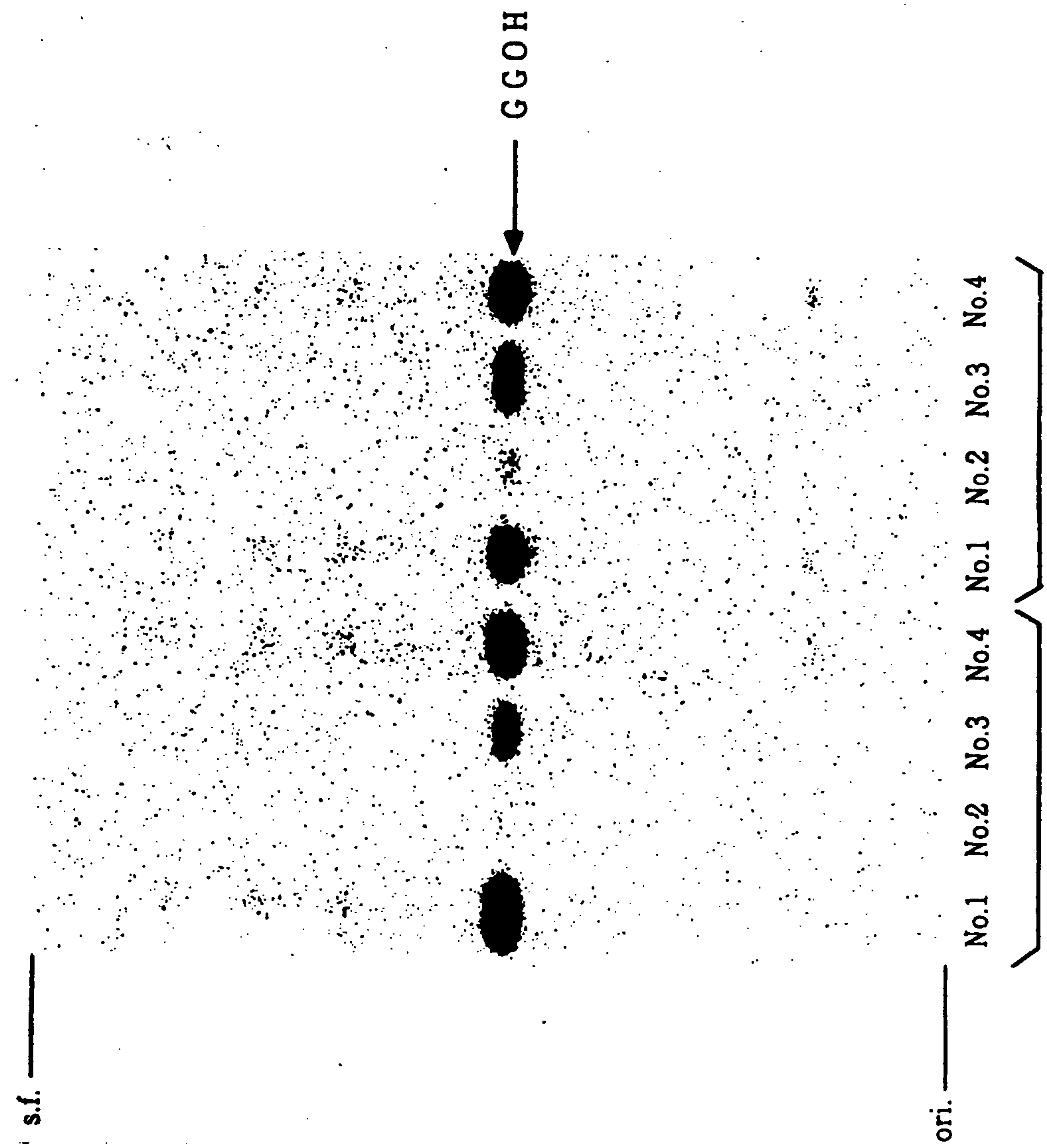
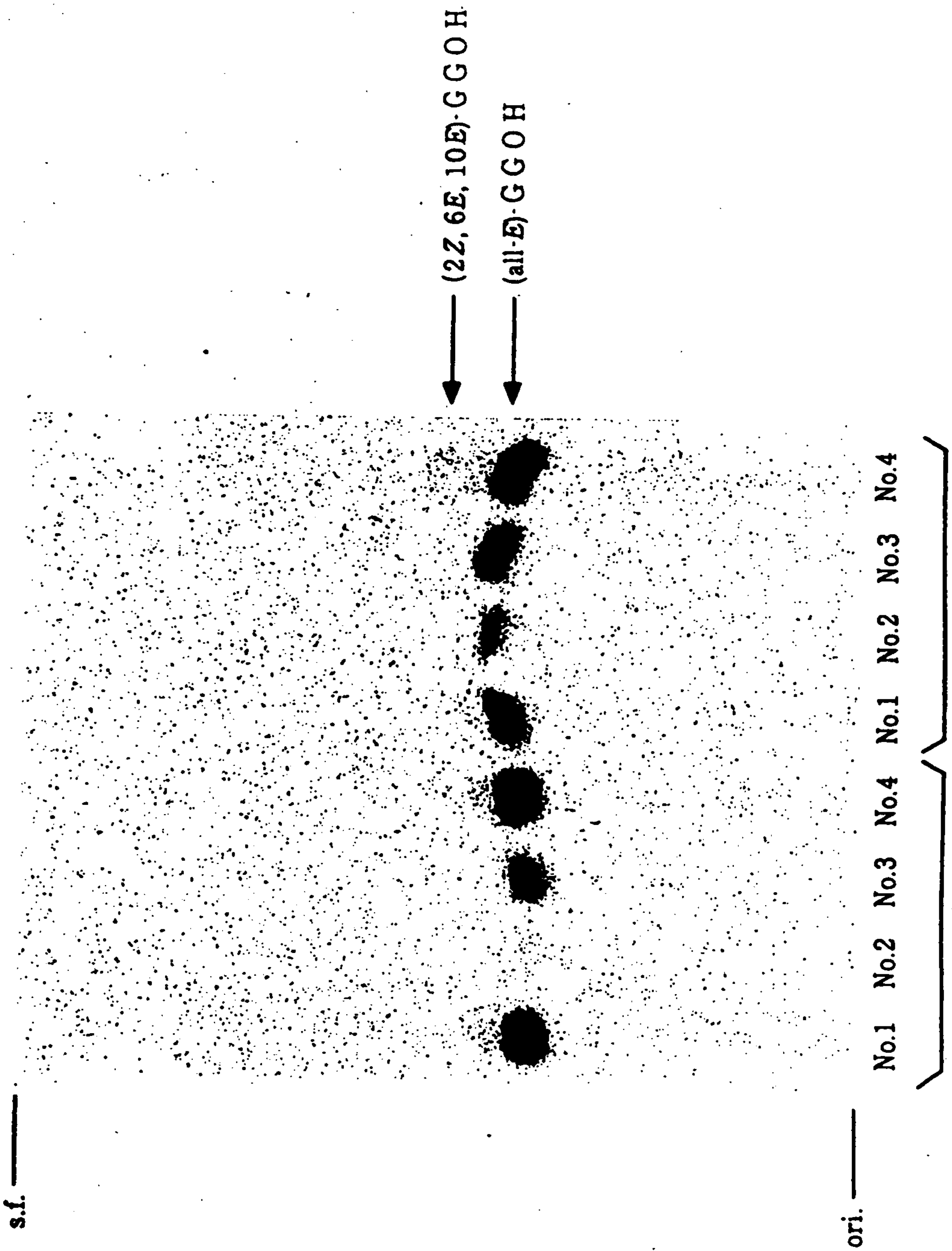


Fig.9


(2Z, 6E, 10E)-GGOH
(all-E)-GGOH
s.f.
ori.
No.1 No.2 No.3 No.4
pTV118N
No.1 No.2 No.3 No.4
pTrc99A